ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1971
PART II

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

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NATIONAL CANCER INSTITUTE

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

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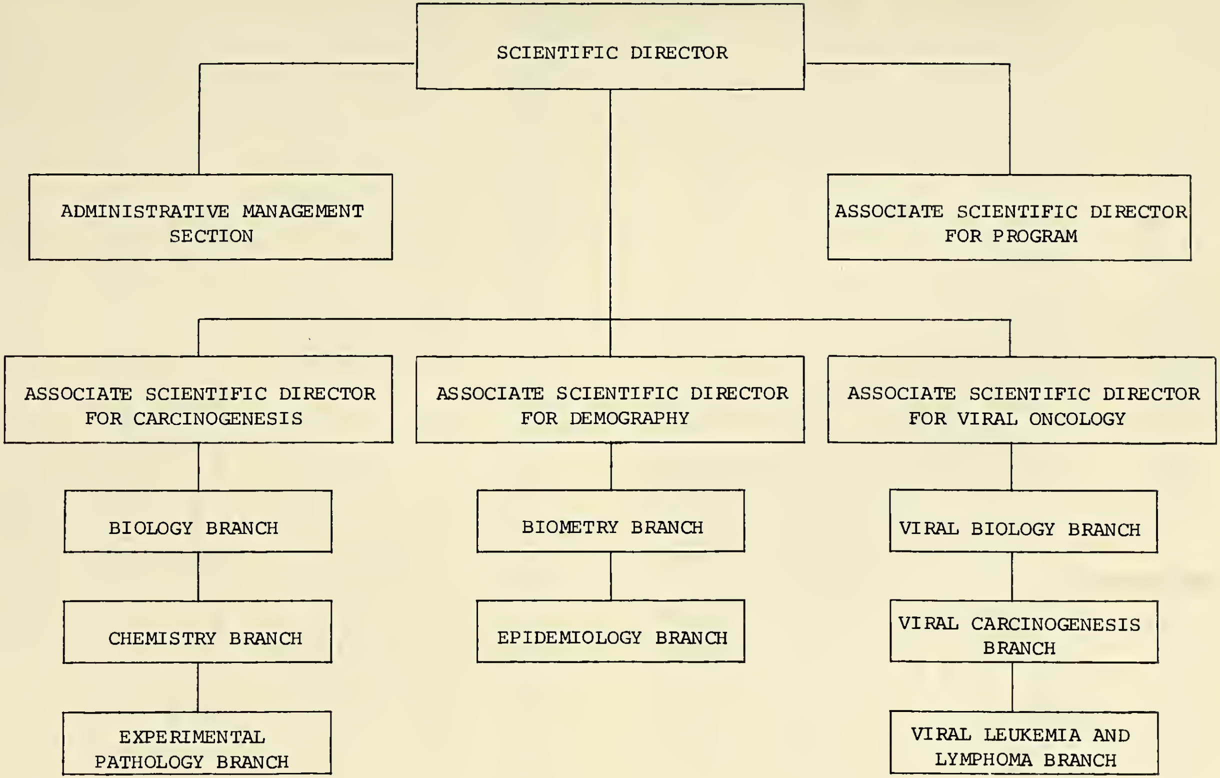
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SUMMARY REPORT
ETIOLOGY AREA - NATIONAL CANCER INSTITUTE
July 1, 1970 - June 30, 1971

For the last three years the summary report of the Scientific Director for Etiology dealt with general aspects of the total Etiology program. The report set forth goals and objectives of the Area, a conceptual base upon which a broad attack on cancer etiology and prevention could be developed, program approaches to be used for attaining the objectives, and indicated the magnitude of the task and the investments required. Since these features were set forth in broad perspective and within a fairly long time frame, the material contained therein is still germane, and will not be repeated here.

A simplified synopsis of major goals, and of pathways to them, is given in Figure 1. More detailed program logic networks for Demography, Carcinogenesis and Viral Oncology will be found under specific area presentations.

All activities in Etiology are directed towards cancer prevention. Research output is continually utilized in resetting more advanced priorities and in formulating those activities that offer the highest promise of success. The major structures of this scientific management process are indicated in Figure 2.

MAGNITUDE OF THE PROBLEM

Cancer occurs in all parts of the world, but variation in the incidence of specific forms of cancer is the rule rather than the exception. A range of ten or twenty-fold is common and for some types of cancer it is far wider.

In general, the rate of cancer mortality in the United States occupies an intermediate rank in the worldwide range, but the rank of mortality from specific types of cancer varies markedly. The United States white population has the lowest mortality from cancer of the stomach and close to the highest from cancers of the colon and female breast. As shown in Table 1, the incidence rates of various cancers in the U. S. are expected to increase from a low of 25% each for leukemias and breast cancer to a high of 180% for cancer of the pancreas. This table also presents a synopsis of leads towards identification of etiologies.

Available evidence suggests that environmental agents and social practices, rather than genetic factors, are largely responsible for variation in the occurrence of cancer in different populations. It should therefore be possible to reduce the occurrence of the major types of cancer in the United States to the level of the lowest ranking country for that type. Such a reduction would cut mortality from cancer by one-third. This is the minimum goal we should aim for.

More than 52 million Americans now living will eventually have cancer (one in four persons) according to present rates. Cancer will strike over the years in approximately two of three families. In the 1970's in the United States alone, there will be an estimated 3.5 million cancer deaths, 6.5 million new cancer cases, and 10.0 million under medical care for cancer unless the present trends are moderated. Such trends mean that in 1976 there will be 346,000 cancer deaths and 747,000 new cases and in 2000, 471,000 cancer deaths and 1,085,000 new cases.

In 1971 there will be 335,000 cancer deaths in the U. S. or one cancer death nearly every one and one-half minutes. There will be 635,000 new cancer cases and 975,000 Americans will be under care for cancer. The last full study on economic cost of cancers in the United States in 1962 estimated an annual cost at that time of $11 billion. About one-half the cancer cases are below age 65; life expectancy and economic earning power are reduced over a considerable portion of the economically productive ages.

Although cure rates for cancer patients have steadily improved over the past several years, the mortality (both in actual numbers and in rates corrected for population growth and other changes) continues to rise. This is because new cancer cases are appearing at an even greater rate than the improved rate of cures. Some kinds of cancer are increasing faster than others; by the year 2000, if present trends continue, cancer of the lung will have increased 52 percent, large bowel 40 percent, leukemias 25 percent and pancreas 180 percent to an overall total of 1.2 million new cases per year.

PROGRESS, PROBLEMS AND OPPORTUNITIES

A. DEMOGRAPHY. Cancer can be prevented by identifying its causes and: (a) removing them, and/or (b) increasing the body's defenses against them.

Thus, it is important to know not only what causes cancer, but also who is unduly susceptible or resistant. Susceptibility may be immense. For example, if an identical twin develops leukemia during the first year of life, his co-twin is virtually certain to develop leukemia within weeks or months. This increased risk fades quickly with age, and entirely disappears by 6 years of age.

Recently there has been a substantial increase in our list of characteristics of persons who are unusually susceptible to leukemia, lymphoma and other cancers. Once such a list has been created, it can be examined for a common denominator. Many items can be explained by a known or possible genetic defect, sometimes extensive enough to cause various abnormalities in the appearance of chromosomes. It should be noted that the only environmental agent known to be leukemogenic in man, ionizing radiation, and the strongest suspect among the chemicals, benzene, produce long-lasting chromosomal abnormalities in somatic cells.

By contrast, persons at high risk of lymphoma (solid tumors of lymph tissue) have inborn immunological deficiencies (ataxia-telangiectasia, Wiskott-Aldrich syndrome, or congenital x-linked agammaglobulinemia). Consistent with these observations is the increase reported in the occurrence of lymphomas among persons given immuno-suppressive drugs following renal transplantation. A sudden upturn in one form of lymphoma, Hodgkin's disease, at about 8 years of age, also suggests a relationship to immunological development. Normally, at this age, there is a marked spontaneous involution of lymphoid tissues throughout the child's body. Lymphoid tissues are centers of immunologic activity. It is tempting to think that some failure in this normal regression accounts for the upturn in the occurrence of the neoplasm.

There are many other inborn defects that carry high risk of cancer of various types. In the aggregate, they do not account for a large proportion of cancer but they probably do constitute a large percentage of cancers among young people. In this group, prevention or early detection and treatment can provide decades of healthy life. Peaks soon after birth in the frequency of diagnosis of leukemia, neuroblastoma, Wilms' tumor, and certain forms of brain cancer indicate that many of these neoplasms are likely to have been initiated during intra-uterine life.

Knowledge of these relationships permits physicians to examine children with certain defects more closely than usual, since early detection and prompt removal of these tumors can be life-saving. Scientists, on the other hand, can explore the origins of the tumors in the light of what is known of the associated birth defects. Thus, instead of studying a tumor in terms only of itself, new avenues of approach have been opened. What is learned through these rare mistakes of nature may well apply to prevention of large categories of cancer. In particular, the epidemiologic identification of individuals and populations at increased risk of different cancer patterns should aid the investigator in virology and chemical carcinogenesis in searching for specific etiologies or combinations thereof.

During the past year the following activities were begun or increased in emphasis:

(1) Migrant Studies - Theories of Cancer Etiology. The migrant studies have triggered extensive examination of some of the factors that may lead to colon cancer, involving laboratory studies and the examination of populations in different parts of the world. The epidemiologic evidence in colon cancer has led to an initiator-promoter model which is capable of being tested by collection of additional observations in man. A similar consideration of stomach cancer would imply that some very early events (before the age of 15) operate as initiators. For colon cancer the initiator would come later in life. The notion of early events having important effects much later in life is being studied with respect to breast cancer where there is now some evidence that hormonal status within the first decade after puberty may be of considerable importance in the later development of breast cancer.

(2) Data Analysis - Death Certificates. A study of death certificates of all persons who died of cancer in the United States over an almost 20 year period, in addition to providing information on geographic clustering of certain forms of cancer, indicates a large increase in cancer mortality in the non-white population, over time. Negroes appear to be

at particularly high risk of cancers of the respiratory system and the upper alimentary tract. The rate of increase of lung cancer among females is now greater than that for males. We should see a sharp increase in deaths from lung cancer among women over the next few years.

(3) Third National Cancer Survey. The TNCS is now aiming toward a preliminary report of incidence data by late Fall 1971. The emphasis in the survey has been on completeness in case reporting. It now appears that the Survey should cover two years, rather than the three originally planned. The collection of data on the "rare" sites could continue for a third year.

The TNCS will provide a firm base for further work. For example, it will be possible to follow a sample of cases over a period of years to identify episodes of hospitalization, and establish events of medical and economic importance subsequent to diagnosis.

(4) End Results Program. The End Results data have pointed up the long survivals possible for patients with tumors showing no evidence of spread beyond the site of origin. Compared to persons of the same age without cancer, 20-year survival for this type of cancer patient is 85% for cancer of the uterus, 75% for ovary, and 60% for colon, rectum, breast, and bladder.

(5) Animal Cancers. Unusual aggregations of disease have been seen among domestic animals with malformations differing markedly among species. These observations are useful for both etiology and therapy studies, since the affected animals can be tested in ways that might be considered inappropriate in man. In addition, the veterinary registers are identifying high risk groups that point toward similar studies in man.

(6) Industrial Epidemiology. The cancer risk to steel workers, smelter refinery workers, uranium miners, some white collar occupational groups, and 6500 U. S. Army World War II male radiology technicians has been examined. No form of cancer, including leukemia, was found in excess. In addition no genetic effects of radiation were discovered in their children.

In view of importance of the industrial environment as a possible source of cancer, the NCI will become more heavily involved in industrial epidemiology.

B. CHEMICAL CARCINOGENESIS. The observed relation between exposure to certain chemicals and the development of cancer in man has been historically the first step towards knowledge of the etiology of cancer. A listing of agents presently known to produce cancers in man includes some 10 specific chemicals, 12 mixtures and crude products and, in addition, radioactive materials and radiation (including x-rays and ultraviolet light). The number of items is relatively small because studies capable of identifying an agent as directly causative of cancer in man require large-scale and time consuming epidemiologic studies and have been conducted for a very small proportion of the potential exposures of man. In fact, essentially all materials that have been demonstrated to be carcinogenic for man have also been found to be carcinogenic in animals.

Most chemical carcinogens known from animal studies appear active in a variety of animal species. It is estimated that more than 1000 chemicals have so far been found carcinogenic in animal bioassays out of about 6000 that have been tested.

The major types of cancer in man that can be directly related to known carcinogenic exposures are as follows: (a) cancer of the lung related to cigarette smoke and certain other inhalation hazards (particularly asbestos, chromates and radioactive materials); (b) cancer of the skin related to exposure to a variety of crude tar products and combustion products as well as to ultraviolet light and other forms of radiation; (c) cancer of the bladder related to exposure to aromatic amines and their derivatives; (d) leukemias related to exposure to radioactive materials; and (e) mesotheliomas related to asbestos exposure. A number of other human cancers have been considered to be related to exposure to environmental carcinogens but the evidence is less clear.

Because of the widespread use of some of these materials, the proportion of the total population exposed and affected is extremely difficult to estimate, with the exception of such materials as cyclamates, DDT and tobacco smoke. Since it has been estimated that the majority of the present cases of lung cancer in the United States are attributable to cigarette smoking, that factor alone appears to be responsible for a death toll in the neighborhood of 40,000 deaths a year. Current projects to produce a less hazardous cigarette must therefore be expanded

The development of our knowledge on the causative factors of cancer in man can be projected along the following lines: (a) epidemiologic studies to correlate specific exposures to certain types of cancer in man with particular emphasis on occupational exposures; (b) additional bioassays in animal models which will identify chemicals as carcinogenic in animals and therefore potentially carcinogenic in man; (c) studies of animal human correlations by comparisons of bioassay results with the results of epidemiologic studies or by bioassays on isolated human tissues by _in vitro_ techniques; and (d) by studies of pharmacologic and metabolic correlations among species.

The major areas of opportunity and need for progress in cancer prevention through studies in Chemical Carcinogenesis are: (a) develop better means for the removal of hazardous chemicals from the environment; (b) develop means to enhance the ability of the host to detoxify environmental agents; (c) determine the extent to which chemicals act in synerigistic concert with viruses or with physical carcinogens such as irradiation; and (d) develop more rapid and more sensitive means to detect and bioassay the cancer-inducing effects of chemicals for man. The latter area of activity is particularly important. For example, it is estimated that approximately 200,000 new chemicals enter our environment each year. If only 500 to 2000 of these chemicals enter at reasonably high levels, and if only 10% of these are hazardous, then at least 50 to 200 new bioassays must be conducted per year. With present assays it takes approximately 2 years with 200 mice at approximately $200 per animal to test 1 chemical. It is hoped that newer studies in animal and human tissue cultures such as those being conducted by Drs. DiPaolo, Heidelberger, Sachs and others will diminish the 2 years and $40,000 now required to test a single compound.

During the past year the following specific activities were begun or increased in emphasis:

(1) The Tobacco Working Group of the Lung Cancer Task Force was instituted with the mandate of identifying the criteria and prescribing the methods for the development of a less hazardous cigarette. Initially, the characteristics of tobacco and smoke were defined through the examination of agronomical and industrial practices, and the analytical mapping of chemical components. Simultaneously, a consensus was reached on the design of bioassay methods in laboratory animals, to monitor the carcinogenicity of smoke itself. Protocols for tobacco characterization, chemical analysis and bioassay have resulted. With the definition of updated experimental methods, the Tobacco Working Group devised an optimum experimental design for the development of a less hazardous cigarette, and recommended the testing of several cigarette types, representing modifications with a promise for a less hazardous product. Research and development contracts were awarded to implement the recommendations, with preliminary results expected in early 1972.

Plans for additional research include (a) development of inhalation bioassay for small rodents, (b) testing of artificial tobacco substitutes (cellulose derivatives etc.), (c) testing of low nicotine-low wax tobacco varieties, (d) development of drugs that may help discontinue the smoking habit (nicotine antagonists, nicotine antibodies, etc.), and (e) biometric surveys to determine real smoke intake doses in man.

(2) Bioassay. A new contract at the Stanford Research Institute directed at establishing a ranking of suspicion of carcinogenic hazard for compounds present in man's environment, will allow the best utilization of current limited bioassay resources and may provide early guidelines to the chemical industry, as to the hazards that may be expected by the introduction of a new compound.

The significance of current bioassays for carcinogenicity derives mainly from two considerations, namely, that (a) humans in general react like other animals to a variety of insults, the differences being usually quantitative rather than qualitative and (b) current bioassay methods, even when performed with adequate biometrical and qualitative controls, can be expected to detect only those compounds that are strongly carcinogenic.

These two reasons prescribe that the greatest attention be given to positive results in current carcinogenicity tests.

Testing and retesting of environmental chemical pollutants at NCI continue to include agricultural chemicals such as pesticides, artificial sweetners such as cyclamates and saccharin, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Food preservatives are also under constant scrutiny. Recent findings suggest that human digestion and storage of fish and meat products preserved with NaNO2 might produce carcinogenic nitrosamines. An investigation by S. Mirvish revealed that nitrosamine formation may occur in food stored under mildly acidic conditions. Two industrial chemicals widely used in consumer goods have been shown to be carcinogenic: propylene imine, produced in large quantities and used in a variety of solvents and adhesives, and propane sultone, produced in small quantities and used as a solvent in printing inks, dye pastes for printing yard goods, etc.

(3) Development of Bioassay Models. Among the more recent accomplishments are (a) a method for the induction of bronchogenic carcinomas by intratracheal instillation: (b) inhalation chamber techniques; (c) a method for induction of glandular stomach cancer by administrations of low-protein, high-salt diets with carcinogens; (d) induction of urinary bladder cancer in hamsters by feeding aromatic amines; (e) a model for the determination of host-mediated mutagenic effects of carcinogens in neurospora; and (f) development of *in vitro* studies of neoplastic transformation by chemicals.

(4) Identification of Carcinogenesis Processes. Studies on the microsomal enzyme have indicated that these are responsible for the hydroxylation and activation of polynuclear hydrocarbons carcinogens. Thus, an enzymatic basis for the covalent binding of polynuclear hydrocarbons to DNA has been discovered.

C. VIRAL ONCOLOGY. The probability is very high that viruses responsible for at least some human and domestic animal cancers have been found and that it will be possible to develop measures (including test vaccines) for the prevention or control of these diseases.

There are now over 100 viruses which are known to cause virtually all kinds of cancer in every major group of animals including sub-human primates. Many of these viruses will not only replicate, but will induce "cancerous" transformations in human cells grown in tissue cultures. The direct application of this laboratory know-how to human disease has revealed that virus particles of 3 types can be detected and in some cases isolated from patients afflicted with different types of cancer--Type C, Type B and herpes Type (HTV). Particles of one kind (Type C) are identical with those known to induce leukemias and sarcomas in laboratory animals. Current studies by Huebner and others have culminated in one of the most exciting, unifying hypotheses of cancer etiology yet postulated.

A unifying hypothesis of cancer etiology. The highly predictable natural incidence and behavior of most cancer in animals as well as in man, plus a number of new discoveries and the resulting development of new concepts and insights, led some investigators to concentrate on the unique C-type RNA tumor virus genome as the most likely important viral cause of the generality of cancer. These new concepts and approaches, and the results of studies carried out in 1970 were epochal, since they led to a testable (heuristic) new hypothesis concerning the basic inherited nature of the RNA viral genome as a general cause of cancer and to a unitary theory capable of explaining spontaneous cancer as well as cancer evoked by exogenous environmental pollutants, endogenous physiological aberrations, genetic defects and mutations, as well as cancer clearly induced by viruses in experimental animals.

The hypothesis of Huebner and Todaro, et al. proposes that cells of probably all vertebrates have RNA tumor virus genomes of the C-type. These genomes are postulated to be transmitted from parent to offspring, and from cell to daughter cell, as part of normal inheritance. Host regulator genes and the repressors and various environmental carcinogens are regarded as factors controlling the expressions of the oncogene(s) and virogene(s) of the generally switched off viral genome. This hypothesis therefore views cancer as a natural biological event determined by spontaneous and/or induced "switch on" or derepression of universally prevalent specific viral oncogenes, thus providing a possible basis for a unifying theory of cancer which is consistent with naturally occurring cancers, as well as with those induced by radiation, chemicals and viral agents. Genetic defects, mutant genes, inducing agents, and finally the aging process itself, all appear to act to decrease the repression of the endogenous virogene(s) and oncogene(s).

Intrinsic enzymes of RNA "Tumor Viruses". A new series of findings which, among many other things, may contribute to the identification of a "switch" for the Huebner hypothesis are the detection and characterization of performed enzymes in RNA tumor viruses.

Genetic expression in bacterial cells is known to be mediated by transcription of the genetic information, coded in the form of base sequences in DNA, into RNA molecules followed by translation of the RNA into specific proteins. Until recently the operation of a similar mechanism in mammalian cells was unquestioned. In recent months Temin and Baltimore independently reported that RNA-containing oncogenic viruses contain a polymerase enzyme, which transcribes the genetic information contained in the RNA of these viruses directly into DNA. This finding, and others, have been fully confirmed and extended by Spiegelman et al. and Green et al. who have shown that the DNA formed is complementary to the viral RNA. More recent studies have revealed the presence of this enzyme in visna virus and in several simian foamy viruses, (Todaro, unpublished) but not in approximately 10 other non-oncogenic RNA viruses of animal and tumor origin. Of further interest and apparent importance are preliminary reports by Spiegelman et al. and Gallo (NCI) that this polymerase was present in peripheral leukocytes from 5 leukemic patients but not in cells from 10 normal persons.

These findings are important because this unique mode of replication of oncogenic viruses, through a RNA-DNA hybrid molecule, provides the possibility of selectively interfering with this step by chemotherapy, utilizing components which may interfere with DNA-RNA hybrid formation. Thus, new avenues for the chemotherapy control of viral produced cancer are opened. The polymerase enzyme can be utilized in the laboratory to prepare the DNA complement of the RNA-containing oncogenic viruses. This complementary DNA has been shown by Spiegelman to bind with high selectivity and form a DNA-RNA hybrid with the RNA of the oncogenic virus. Thus, the DNA made in the laboratory can be used for virus detection.

A second category of viruses is of the DNA type of which the Epstein-Barr virus (EBV) is one example. EBV is a herpes virus but is antigenically different from known agents of the herpes virus group, and antibody evidence of infection by it is widespread in human populations throughout the world as well as in other higher primate animals. Such evidence is present in 100% of children thus far studied, with a peculiar type of lymphoma (Burkitt) highly prevalent in certain regions of Africa, whereas the specific antibodies are found, in much smaller amounts, in less than 50% of nondiseased children of similar age in the same regions. A very high incidence of antibodies to this virus is also found in patients with postnasal carcinoma, and in 100% of individuals who have had infectious mononucleosis. Other evidence strongly indicates that this virus is a major cause of infectious mononucleosis, a non-malignant human disease in which cells of the same type that are involved in leukemia proliferate rapidly and behave for a short while as if cancerous. This raises the question whether the same virus might not also cause the cancers with which it is highly associated, under certain host circumstances or in joint interactions with other viruses or chemical agents of the environment.

These and many other data strongly suggest that virtually all attainable evidence is at hand to incriminate EBV as an oncogene for man. The question however of whether a virus can ever be proven to be oncogenic for man is of course very difficult and probably not answerable within our society. The only direct means of proof is not available to us as the inoculation of the newborn of our species will not be done. Consequently, studies on the proof of oncogenicity seem to be diverting to the attainment of enough predictive information to prepare and conduct a field trial with preventive means, such as that containing the candidate virus within a safe and effective vaccine. As with rabies and other viruses of man, proof of etiology is surmised through effective prevention with appropriate inocula containing the specific organism. In regard to infectious mononucleosis, Burkitt lymphoma, nasopharyngeal carcinoma, and perhaps to some forms of Hodgkin's disease and chronic leukemia this approach may be feasible in that EBV seems clearly to be a horizontally transmitted agent, and therefore amenable to classic vaccine approaches with the particulate pathogen.

So then with these formidable deterrents and considerations is it possible to devise criteria upon which one can reliably assign a role of oncogenicity for any candidate virus? If it is agreed that this can never be done directly, then indirect pieces of information must serve this purpose. With EBV, I believe the data are at hand to firmly assign a role of oncogenicity for man. Viruses which induce tumors do two things that other nononcogenic viruses do not do:

(a) They transform cells. This phenomenon which can be measured in vitro or in vivo is defined as morphologic alteration with prolonged life.

(b) Since cancer is an inheritable disease (from cell to daughter cell), one must therefore assume that any oncogene has the capacity to reprogram cellular genome, in the sense that following exposure the cell produces something or does something that it would not have done prior to exposure. The induction of neoantigens on plasma membranes through exposure to EBV indicates that this virus does indeed have the capacity to command the cell to make a new protein in an inheritable way that it would not have done without exposure to the virus. So far as is known, viruses not known to have an oncogenic capability do not have this capacity. In a genetic sense the probability of EBV as an oncogene is further strengthened by its apparent major etiologic role in infectious mononucleosis and philosophically by the induction of neoplastic disease in rabbits, chickens, monkeys, and frogs by other herpes viruses.

A similar case seems to exist for possible involvement in human cancer of one of the previously known members of the herpes virus group (herpes simplex type 2), which has been found to be highly associated with cervical and perhaps penile cancers. The peculiar epidemiology of this cancer suggests that it may be caused by a venereally transmitted agent of which herpes type 2, through studies of Rawls, et al. and Josey, et al., is one requiring further research. In this regard, and most recently, Aurelian, Johns Hopkins University, has presented convincing evidence that cells of earliest lesions, including dysplasia and carcinoma in situ, contain immunofluorescent virion antigen, whereas apparently normal adjacent cells do not.

BREAST CANCER STUDIES

Until recently, research activities in breast cancer have been limited to exploratory studies in search of evidence that viruses might be associated with human breast cancer. The consistent finding by electron microscopy of particles resembling the known RNA tumor viruses in human materials, and the successful isolation and propagation of three candidate viruses -- one of subhuman primate (Rhesus monkey) and two of human origin -- have led to the supposition that viruses of this type should be considered as major oncogenes for animals "higher" than the mouse.

Isolation of a Virus From a Monkey Breast Cancer. The most advanced and one of the most significant developments in breast cancer is represented by the discovery, isolation, and successful propagation of a C type virus associated with an adenocarcinoma of the breast of a Rhesus monkey. It has been designated as the Mason-Pfizer Monkey Virus (M-PMV). This is the first C type virus of any primate thus far propagated in sufficient quantities to support systematic investigations on its virological, biological, immunological, chemical and physical characterization. The M-PM virus grows readily in primary Rhesus monkey embryo and fetal lung cultures, and to a lesser extent in cell lines of chimpanzee lung, human embryo and human lymphoblasts (NC-37).

The M-PM virus was shown by Chopra, et al. (NCI) to form from precursor intracytoplasmic A type particles which bud through the cell membrane as in the formation of B particles of mouse mammary tumor virus, but not to have spikes characteristic of the latter. The mature particle is indistinguishable from C type particles in ultrastructural morphology although the nucleoid does not form during budding, at cell membranes, as do all of the other known C type viruses. The M-PM virus therefore possesses certain characteristics of both B and C type viruses. The nucleic acid is of the RNA type and the virus particles contain RNA-dependent DNA polymerase as do all known oncogenic RNA viruses thus far studied (Spiegelman).

Demonstration of an RNA virus in Cell Lines Derived from Human Breast Cancer.

Two groups of scientists have demonstrated morphologically similar RNA viruses in tissue culture lines derived from two different cases of human breast cancer. The former investigators also reported isolating the same type of virus from two cases of cancer of other types, among a total of 56 specimens from human cancer patients studied.

The virus produces no CPE or other morphological evidence of its presence, but it can be detected both by ^{3}H-uridine incorporation and by electron microscopy. The virus is found in appreciable amounts in tissue culture fluids, and can infect other human cell lines. It resembles the mouse mammary tumor virus (MTV) in that it has peripheral spikes of identical size, spacing and morphology but it is only 2/3 the size of the MTV, i.e., 50-75 mμ as compared with 100/140 mμ. Except for its slightly smaller size, therefore, it resembles the only presently known breast cancer virus (MTV).

Moore and associates have reported finding particles resembling the B type virus of mouse mammary cancer in 5/75 (6.7%) milk specimens from normal women in the Philadelphia area. In further studies as yet unpublished, much higher frequencies of similar particles have been found in milk from women of special or high risk population groups.

No human candidate breast cancer virus is yet available in sufficient quantities to support seroepidemiological studies, but attempts to scale up production of one of them are underway. The same bank of specimens used in the M-P monkey virus tests are on hand to be tested similarly with the human agents as they become available.

DISCUSSION

Between 30 to 90 percent of cancer deaths in man are preventable. Mortality statistics from around the world show that if cancer mortality in the United States, site for site,was comparable with that of the lowest country reporting for each site, approximately 100,000 lives would be saved each year. The major sites in which gains should be made are cancer of the colon and rectum, cancer of the lung, breast cancer, cervix and uterus (particularly among non-whites), cancer of the ovary, cancer of the prostate, and probably leukemia.

Information is already in hand to reach these goals for lung cancer and for cancer of the cervix and uterus. These 2 sites alone would account for 60,000 or more savable lives. A concerted research effort is called for in cancer of the colon, breast cancer and cancer of the prostate. There is promise of substantial pay-off in these sites. For example, migrant studies have provided very strong evidence that cancer of the colon is a disease with a very strong environmental base. Japanese migrants to Hawaii show a 3 to 5-fold increase in contrast to Japanese on the home islands. When we find those things in the new environment that lead to so large an increase, application of this knowledge to the present United States population could reduce the U. S. incidence by another 25-30,000 lives a year.

Thirty-percent (or 100,000 lives) is a minimum estimate. If we add to the preventable cases (in terms of current knowledge, or knowledge that should be available in the next few years, earlier diagnosis and improved treatments--where prevention has broken down), this number should be easily doubled. For example, if the most recent results of the HIP-NCI (Health Insurance Plan-National Cancer Institute, unpublished) studies on early diagnosis of breast cancer were applied across the country, we might anticipate 12,000 fewer deaths each year from breast cancer alone. If the finding that early pregnancy (before age 20) cuts in half the probability of developing breast cancer, could be translated into a prophylactic treatment for young women (i.e., create the hormonal milieu of pregnancy without actual pregnancy) another substantial reduction in breast cancer deaths would follow.

Current work on identifying the cancer "susceptibles" as successful will lead to cutting down cancer deaths 2 ways. First, by identifying susceptibles, we will know what people to watch closely and regularly, so that we might diagnose their disease early, and thus treat it successfully. Second, by identifying the cancer susceptibles, it should be possible to protect them from those environmental assaults which could be carcinogenic to them -- while essentially innocuous to others.

Ninety percent reduction in cancer deaths could be reached within the present century, if, in addition to current knowledge on prevention, and universal application of the best diagnosis and treatment techniques, our research uncovered (a) the environmental elements that cause migrants' cancer rates to change toward those of their new countries; (b) those environmental elements which lead to different rates in different countries in the world; and (c) the susceptibles whom we must and can protect.

While there are major problems in the prevention of virus-induced cancers, not heretofore experienced in other areas of infectious disease, we should anticipate substantial inevitable success in the control (including prevention) of these diseases in man. These problems include the probable difficulties in using conventional vaccines for prevention if a major means of virus exposure is vertical rather than horizontal. Secondly, the "peaking" phenomenon of acute lymphocytic leukemia in Caucasian children at about 4 years of age and other similar phenomena suggest that it would take 5-10 years before the results of a preventative field trial were known. Even with a "clustering" disease of relative high incidence, such as Burkitt's lymphoma in equatorial Africa, nearly 1,000,000 children would have to be included in the "treated" cohort observed for 5-8 years to determine whether the expected 40-50 lymphomas would be eliminated or decreased.

These difficulties notwithstanding, opportunities for cancer prevention and control are very bright through application of existing knowledge and through a prioritied commitment to seek new information with innovative application for man.

A Note on Management - The obligatory opportunity to plan and manage biomedical activities from basic or fundamental research upwards through development and application, with effectiveness and efficiency, requires skillful staff and line personnel of unabashed motivation, objectivity, compassion and tact. Tact is of paramount importance because NCI management must skillfully and with objective credibility persuade constituent scientists to attack specific problems of human disease. We adhere to the belief that NIH exists primarily to allow people to live better and longer and that NCI abets this goal through the conduct of research and development for prevention and treatment of cancer in man. Tact, including the courage and reason to say no, is important also because while the need for widespread program participation is high, many scientists do not understand that even successful completion of their proposed project may not necessarily contribute to the attainment of program goals. This thrust towards the timely control of disease must be tempered with appreciation for and selection of fundamental projects with some expectation of contribution to eventual application to man. This can and should be done through both grants and contracts (funding mechanisms for getting the job done).

We are fortunate to have this admixture of talents and motivation in the Associate Scientific Directors, Drs. Gori, Moloney, Saffiotti and Schneiderman; in the Assistants to the Scientific Director, Drs. Peters, Bryan and Depue, and in the Administrative Officer, Mr. John Patterson. Particular recognition is entirely warranted to their associates whose motivation to program goals clearly exceeds desire for personal recognition.

Refer to summary and individual reports for details of activities within Etiology programs for FY 70.

FIGURE 1

CANCER ETIOLOGY AND PREVENTION PROGRAM

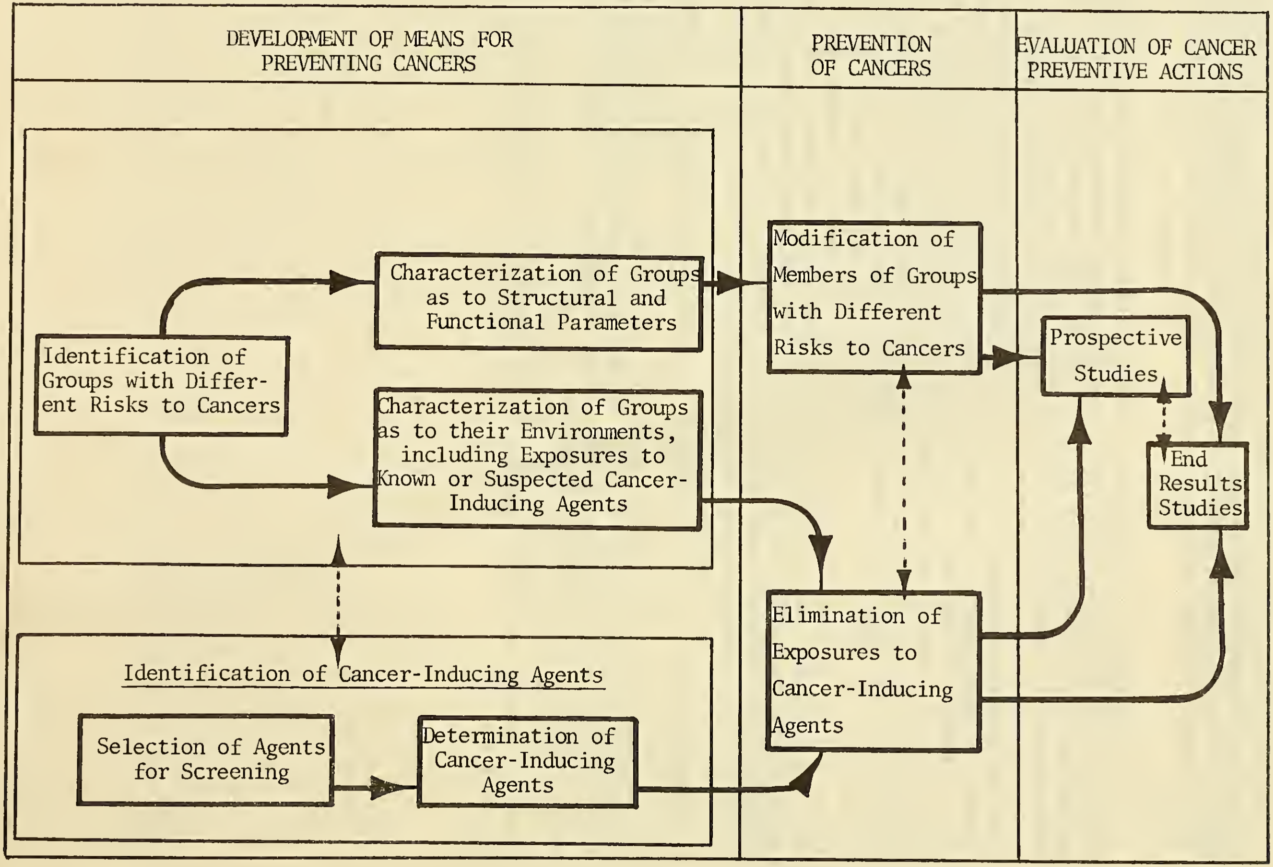
DEVELOPMENT OF MEANS FOR PREVENTING CANCERS
PREVENTION OF CANCERS
EVALUATION OF CANCER PREVENTIVE ACTIONS
Identification of Groups with Different Risks to Cancers
Characterization of Groups as to Structural and Functional Parameters
Characterization of Groups as to their Environments, including Exposures to Known or Suspected Cancer-Inducing Agents
Identification of Cancer-Inducing Agents
Selection of Agents for Screening
Determination of Cancer-Inducing Agents
Modification of Members of Groups with Different Risks to Cancers
Elimination of Exposures to Cancer-Inducing Agents
Prospective Studies
End Results Studies

FIGURE 2

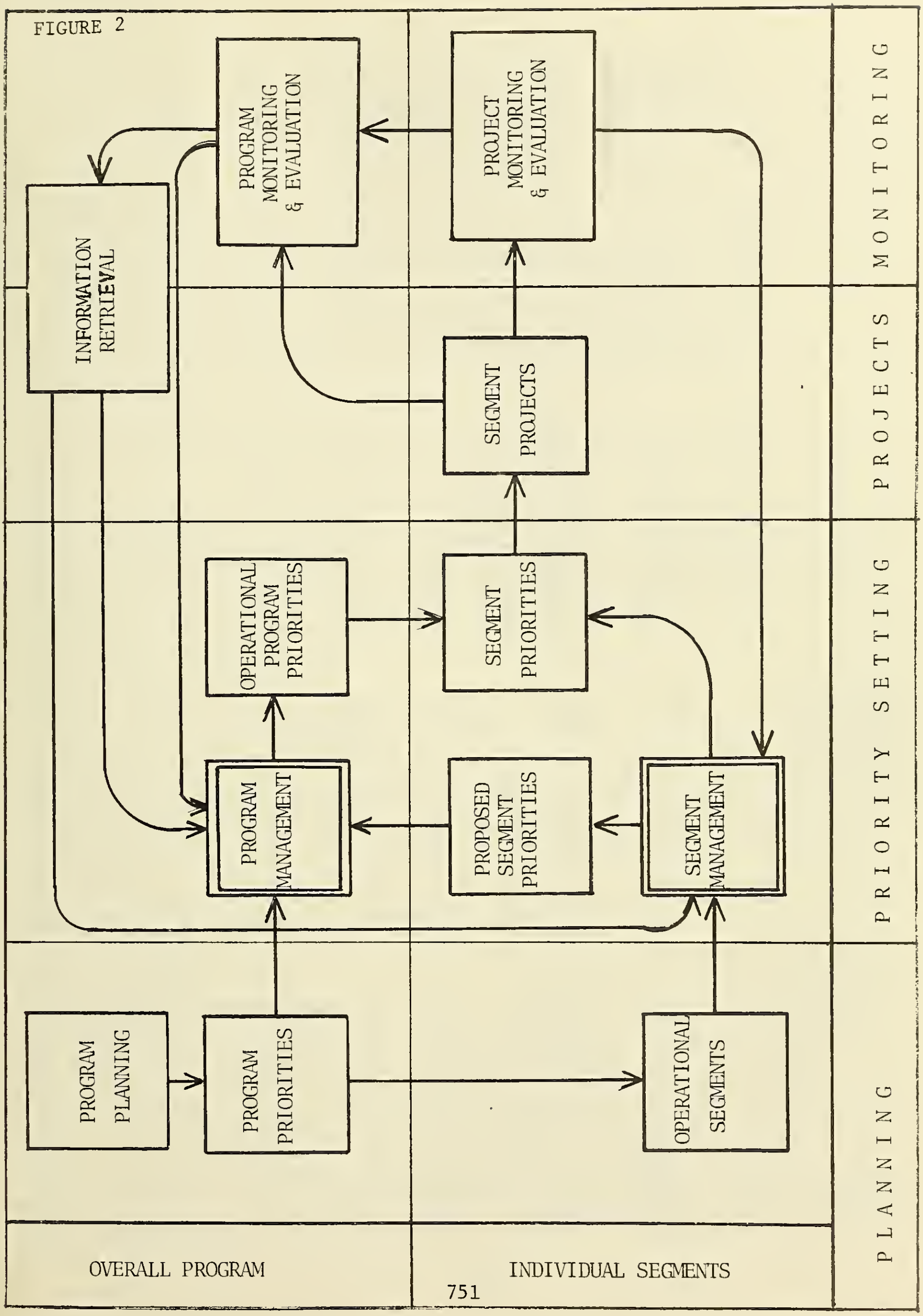
PLANNING
PRIORITY SETTING
PROJECTS
MONITORING
OVERALL PROGRAM
INDIVIDUAL SEGMENTS
PROGRAM PLANNING
PROGRAM PRIORITIES
OPERATIONAL SEGMENTS
PROGRAM MANAGEMENT
OPERATIONAL PROGRAM PRIORITIES
PROPOSED SEGMENT PRIORITIES
SEGMENT MANAGEMENT
SEGMENT PRIORITIES
SEGMENT PROJECTS
INFORMATION RETRIEVAL
PROGRAM MONITORING & EVALUATION
PROJECT MONITORING & EVALUATION

TABLE 1: CANCER INCIDENCE: EXPECTED NUMBERS, 1970-2000; CHANGE IN RATES, 1970-2000; DEATHS, 1970

SITE	NEW CASES (INCIDENCE) 1970	NEW CASES (INCIDENCE) 2000	DEATHS	INCIDENCE YEAR 2000 % CHANGE* IN RATE	MAJOR CAUSATION	MEANS OF PREVENTION
Lung	68,000	144,000	62,000	52	Tobacco Smoke Air Pollution (including on-the-job)	Stop smoking - Reduce pollution Less hazardous cigarette
Large and Small Bowel	75,000	143,000	44,000	40	Intestinal Flora? Heredity? Diet?	Viruses? Other insults. Identify susceptibles and eliminate their exposure.
Breast	69,000	121,000	30,000	25	Virus? Diet? (Hormones) Genetic?	Vaccines. Identify susceptibles.
Pancreas	19,000	101,000	18,000	180	Diet? Virus? Other Insults?	Identify etiology. Identify susceptibles.
Prostate	35,000	61,000	17,000	27	Hormones? Diet?	Identify etiology. Identify susceptibles.
Stomach	17,000	5,000	16,000	(80) decrease	Diet Poor socio-economic conditions	Diet modifications. Sociologic modifications?
Leukemias	19,000	33,000	15,000	25	Viruses. Radiation. Genetic.	Vaccines. Identify susceptibles and limit radiation.
Skin	112,000	186,000	5,000	20	Actinic rays. Genetic.	Limit radiation exposure. Identify susceptibles.
Misc.	120,000	217,000	75,000	30	Multiple	Identify extrinsic and intrinsic factors and modify them.

*Expected change in age adjusted incidence rates. Year 2000 compared to 1970.

SUMMARY REPORT
OFFICE OF THE ACTING ASSOCIATE SCIENTIFIC DIRECTOR FOR DEMOGRAPHY, ETIOLOGY
JULY 1, 1970 - JUNE 30, 1971

The Demography Area, Etiology, is largely concerned with cancer in man. It has three main functions:

a. Uncovering the origins of cancer in man's environment, or in his genetic make-up.

b. Providing consultation and professional support so that other scientists might conduct sound studies, and interpret their data intelligently.

c. Developing basic data on cancer incidence, prevalence, and mortality, their time trends, and their geographic distribution.

Some of these functions lead into interaction and collaboration with laboratory scientists which then bring together the research on animals with the data on man. The two Branches (Biometry and Epidemiology) strongly supplement and support each other. The Epidemiologic Pathology Unit which moved to the Carcinogenesis Area late in the year continues to work in close collaboration with the two Branches.

Public-impact Problems

The Area was much involved in public-impact aspects of cancer in the past year, from surveying data on bladder cancer for a possible association with increased cyclamate use (data so far do not show an increase in mortality beyond the increases due to cigarette smoking - but age distribution and problems of latent period preclude drawing final conclusions), to consulting on the setting of safety levels of radiation, and of food additives, drugs and pesticides (a "socially acceptable" risk level may be attainable), to the possible environmental effects of super-sonic transports.

Migrant Studies - Theories of Cancer Etiology

Some of the long-term activities of the Area have begun to bear fruit. The migrant studies have triggered extensive examination of some of the factors that may lead to colon cancer, involving laboratory studies and the examination of populations in different parts of the world. The epidemiologic evidence in colon cancer has led to an initiator-promoter model which is capable of being tested by collection of additional observations in man. A similar consideration of stomach cancer would imply that some very early events (before the age of 15) operate as initiators. For colon cancer the initiator would come later in life. The notion of early events having important effects much later in life is being studied with respect to breast cancer where there is now some evidence that hormonal status within the first decade after puberty may be of considerable importance in the later development of breast cancer.

Data Analysis - Death Certificates

A study of death certificates of all persons who died of cancer in the United States over an almost 20 year period will shortly appear as an NCI Monograph. In addition to providing information on geographic clustering of certain forms of cancer, and on the aggregations of some forms of the disease, pointing to a common etiology, the major finding in the study is of the large increase in cancer mortality in the non-white population, over time. Negroes appear to be at particularly high risk of cancers of the respiratory system and the upper alimentary tract. The rate of increase of lung cancer among females is now greater than that for males. We should see a sharp increase in deaths from lung cancer among women over the next few years.

Third National Cancer Survey

In addition to death certificates, the Area has two other large data collection systems -- the Third National Cancer Survey (TNCS) and the End Results system. The TNCS is now aiming toward a preliminary report of incidence data by late Fall 1971. The emphasis in the survey has been on completeness in case reporting. It now appears that the Survey should cover two years, rather than the three originally planned. The collection of data on the "rare" sites could continue for a third year. The Survey has suffered from attempts to carry out the original plans with substantially fewer personnel than the original plans called for. The 10 per cent sample of all cases for special studies has not gone as well as hoped for. Physician support for the interviewing program has been lukewarm - if not antagonistic. It will be necessary to explore other ways of developing the financial and related data that the interview program was designed to get.

Since the TNCS represents a substantial improvement over earlier surveys, particularly with respect to data handling and histology, it will provide a firm base for further work. For example, it will be possible to follow a sample of cases over a period of years to identify episodes of hospitalization, and establish events of medical and economic importance subsequent to diagnosis. In addition TNCS provides a base toward moving into etiology-oriented population-based registries. More registries to report cancer under diverse conditions in several regions of the country are indicated, the selection to be governed by: (a) ethnic diversity and (b) the presence of the appropriate talent to investigate leads turned up by the registry data.

End Results Program

The movement toward etiology-oriented registers signals a changed emphasis in the End Results program. If registries can be developed to where epidemiological observations can interact with laboratory experiments by providing information bearing on the choice of animal models, we are likely to advance research on etiology much more rapidly. The End Results program will not discontinue its output describing survival experience of cancer patients, but may have to reduce the number of smaller hospital-based registries contributing this kind of information. Work has begun on End Results Report #4, for distribution at the National Cancer Conference in 1972. For this report a completely new, more comprehensive format will be used taking advantage of the advanced computer capabilities now at NIH.

The End Results data have pointed up the long survivals possible for patients with tumors showing no evidence of spread beyond the site of origin. Compared to persons of the same age without cancer, 20-year survival for this type of cancer patient is 85% for cancer of the uterus, 75% for ovary, and 60% for colon, rectum, breast, and bladder.

Animal Cancers

One area of data collection sometimes overlooked relates to the cancers seen by veterinarians, as reported through cancer registries at various veterinary colleges. Unusual aggregations of disease have been seen among domestic animals with malformations differing markedly among species. These observations are useful for both etiology and therapy studies, since the affected animals can be tested in ways that might be considered inappropriate in man (e.g., the effectiveness of "lump-ectomy" in treating breast cancer). In addition, the veterinary registers are identifying high risk groups that point toward similar studies in man. Finally, they are providing a major source of materials for comparative tumor pathology (man-animal comparisions) by the Epidemiologic Pathology Unit.

Industrial Epidemiology

Much recent work has pointed toward the importance of the environment as a cause of cancer. The Epidemiology Branch has examined the risks to steel workers, smelter refinery workers, and uranium miners, while the Biometry Branch has looked into the risks of cancer in some white-collar occupational groups. Most recently, in collaboration with the Follow-up Agency of the National Academy of Sciences, the Epidemiology Branch examined the cancer history of 6500 U.S. Army World War II male radiology technicians. No form of cancer, including leukemia, was found in excess. In addition no genetic effects of radiation were discovered in their children.

In general, the industrial epidemiology of cancer is one of the weaker areas of the Cancer Institute. With the establishment of the National Institute of Environmental Health Sciences, NCI personnel involved in industrial epidemiology were transferred to NIEHS. Most of these, in turn, have moved to the Environmental Protection Agency. In view of importance of the industrial environment as a possible source of cancer, it would be worthwhile for NCI to become involved once more in industrial epidemiology. Some plans are now being formulated to do this.

Genetics and Cancer

Clearly not all of cancer is of environmental origin. The continued evidence of childhood cancers being associated with congenital abnormalities, the high level of retinoblastoma (2x) and large intestine cancers (4x) in black children compared to whites, and the evidence of increased familial risks in such common cancers as breast cancer point to genetic components of some cancers. In the search for high risk populations, the family clusters have provided a population for extensive laboratory examination for possible cancer "markers."

Consultation

The Area extended its consulting activities to many new cancer-related problems covering much of the research of the Institute and the impact of cancer on the general population. The staff of the Mathematical Statistics and Applied Mathematics Section of the Biometry Branch have been particularly active with in-house staff research. Work has been done with over a dozen branches and laboratories, ranging from problems in histo-compatability testing to the design and analysis of controlled clinical trials under some very restrictive circumstances. Out of the Office of the Chief, Biometry Branch, has come consultation in problems of epidemiology, carcinogenesis, drug safety evaluation, problems of determining safe levels of radiation, problems of tissue transplantation, and the evaluation of new therapies. There has been extensive cooperation and consultation with the Food and Drug Administration, the National Academy of Sciences, the Atomic Energy Commission, the Federal Aviation Administration, and the Veterans Administration as well as with the World Health Organization, and the International Agency for Research on Cancer. Consultation has also been given to the American College of Surgeons, the American Cancer Society. Within NIH, but outside the Cancer Institute, consultation has been given to investigators in NHLI, NICHD, NIAMD, and NIDR.

Problems

Two major problems face the Demography Area. The first is how to manage an increased contract program with little or no increase in professional staff. The second is how to extend the contract program into areas now inadequately covered - especially in view of the shortage of trained cancer epidemiologists in the United States. For example, we are currently unable to entertain development of studies of employee populations greatly desired for the investigation of occupational carcinogenesis. In part, the situation may be ameliorated by development of the prime-contractor concept (or by a parallel development of NCI field research units in the manner of the Medical Research Council Units in Britain).

We have not brought many of the social-science research people into much of cancer research. They should be useful not only in the study of the economics of cancer, the use of medical facilities and in plotting the paths to medical care that could lead to less delay, and thus better prognosis, but also in the study of those cancers which seem to have strong socio-economic components such as cancer of the lung, breast, cervix, stomach, the leukemias, etc.

Despite these shortcomings the future looks very good. We are hopeful that closer collaboration with the laboratory areas will lead to quicker uncovering of etiologic agents, as well as more rapid identification of carcinogens. The childhood cancer, congenital-abnormality studies, and the family aggregation studies show particular promise for providing a base from which to develop laboratory measures of high risk groups. The Third National Cancer Survey will produce important data soon. End Results studies have pointed out important prognostic factors in breast cancer. Finally, we have some very capable young professionals on our staff who should be able to move into positions of leadership as the more senior staff members retire.

Summary Report
BIOMETRY BRANCH
July 1, 1970 -- June 30, 1971

The Biometry Branch has three main functions -- to conduct research on the etiology of cancer in man; to provide statistical consultation and support for other people conducting research; and to provide baseline data against which administrators and researchers can measure their successes (and failures) in reducing cancer incidence and mortality. These functions encompass a wide range of scientific and managerial skills in bringing people and data together.

Third National Cancer Survey

The Third National Cancer Survey continued to be the major data-gathering operation of the Branch during the past year. Field work reached its peak level in 1970 and the initial projection of 75-80,000 new cases per year proved to be correct. The departure of Dr. Bailar in December 1970 to accept a position at the Veterans Administration and his replacement as Survey Director by Dr. Cutler was the occasion for a reexamination of Survey operations. While numerous modifications in the processing of documents has yielded substantial economies, reappraisal made clear the need to emphasize efforts to obtain more complete coverage of reported cases. Present efforts are directed to completion of case findings for the calendar year 1969. This step will facilitate preparation of a preliminary report on incidence data for the first year of the Survey by the Fall of 1971. It now appears that the Survey should cover two years, rather than the three years originally contemplated. While the cutback in survey scope is a matter for regret, a limited job well done is greatly to be preferred to a more ambitious one performed indifferently. One compromise under consideration would be to continue the Survey for a third year for the "rare" sites for which more cases are needed for analysis.

The original Survey plans included interviewing of a 10 percent sample of all cases in several study areas. An appraisal of the interviewing performance is underway to ascertain whether the results warrant continuance or whether modifications, or even discontinuance in some communities, is suggested. Physician support for the interviewing program has been lukewarm, if not antagonistic. Decisions on the future of the interviewing program must be made in the near future.

The scope of the Third National Survey and the emphasis on maximum exploitation of study opportunities elicited many suggestions and requests for special studies, ranging from provision of complete sets of data tapes for a single area to requests for special regional tabulations for diseases presenting regional differences in incidence within the United States. For example, the Academy of Dermatology desires to utilize the information on skin cancer cases from the latter point of view. The Survey may provide data bearing on the hypothesis that Hodgkin's disease involves an infectious disease etiology or represents possible deficiencies in immunologic response which can be manifested differently at different ages in different climates. Some difficulties have arisen in attempting to tap the special study potential of the Survey. The proposal to utilize the Survey to generate sufficient numbers of cases for retrospective studies of such relatively rare sites as male breast cancer was enthusiastically received; however, the sheer mechanics of drafting protocols, securing forms clearance, recruitment of interviewers, and negotiating local arrangements for the conduct of the study has consumed so much time that the Survey may be almost completed before the retrospective case-control studies are underway. Such difficulties reflect problems arising from insufficient staffing. The Survey never received the projected number of required positions. Subsequent events have demonstrated the correctness of the underlying planning and assumptions that the Survey has suffered to a degree from well-intentioned efforts to execute the original plans without the requisite manpower.

On the positive side the Third Survey represents a substantial improvement over its predecessors, in such areas as the handling of histology information. The ability to define more precise site-histology complexes owes much to the recent development and publication of the Standardized Nomenclature of Pathology (SNOP). This ability to identify specific subgroups of cases should lay the groundwork for subsequent special studies, including slide reviews to differentiate epidemiologically distinct and meaningful entities. Another profitable special study is the 10 percent sample of cases for which a detailed medical supplement and abstract of hospital records has been prepared. It appears desirable to follow this sample of cases over a period of years to identify episodes of hospitalization, and to establish a firm description of events, of medical and economic importance, which are presented subsequent to diagnosis.

Twenty years elapsed between the second and third National Survey and it may be twenty years or longer, if ever, before another is attempted. Surveys at such infrequent intervals cannot provide current data on time trends in the presentation of cancers presumably related to changing environmental factors. This requirement can be met only by continuing, population-based cancer registries. Such registries are few in number, the best known being Connecticut and Alameda County, California. More registries to cover diverse conditions in several regions of the country are indicated, the selection to be governed by (a) the ethnic representation and diversity, and (b) the presence of local scientific, technical and medical talent to investigate leads turned up by the registry data. One way to meet this need would be to convert some of the ongoing surveys to continuing population-based cancer registries. Given the criteria enumerated the following four areas merit serious consideration: San Francisco-Oakland, Detroit, Atlanta and the State of Iowa. The Branch staff is currently investigating the possibilities and within the coming year is prepared to develop a statement of the alternatives and the cost-benefit ratios for various options.

We emphasize that population-based registries are a means to an end and that a definition of purposes -- the data to be collected, for whom and for what objectives -- will contribute to the decisions required concerning population-based and hospital registries. These are now being actively reviewed in light of the NCI program needs in the area of cancer etiology.

End Results Program

The existing set of population-based and hospital cancer registries contributing to the End Results program for more than 10 years represents the major U.S. source of data on end results of treatment and cancer incidence. While the program as presently constituted has yielded several important and significant findings, some changes in emphasis seem indicated. Sustained work with the registry materials has pinpointed the need for more population-based registry data for Etiology Area program purposes. This is one driving force underlying a proposed conversion of some National Survey operations in selected localities to continuing cancer registration mentioned above. The switch to population-based registries may lead to reduced support for individual hospital registries contributing to the End Results program. The prospects of maintaining the hospital registry program unchanged, while expanding the population-based registry aspects, appears remote. In addition, the budgetary squeeze on the most important state-wide registries forces a harsh re-examination of the alternatives. The recent reductions in California and Connecticut in support for their cancer registries will force the National Cancer Institute to assume the additional costs; these two registries represent too important a data resource and too large an investment to walk away from them.

These developments underscore the wisdom of the position of the Biometry Branch, consistently maintained over the past twenty years, that individual states should not be routinely encouraged to establish cancer registries. The Branch maxim has always been selective concentration of effort. We have always sought to support work in localities where the most favorable conditions prevailed, particularly where resources for the maximum exploitation of the data potential in terms of special studies could be identified. This principle will attain greater visibility in efforts to establish comprehensive population-based cancer study centers. In the center concept the registry remains an important tool, but a means to an end, justified as a source of leads for other case-control or prospective investigations. Also, the epidemiological observations should interact with laboratory experiments by providing information bearing on the choice of suitable animal models. The two immediate candidates for establishment of population-based centers are the San Francisco Bay area and Hawaii, the former because the caliber of the nucleus of investigators residing in that area and the latter because of the unusual opportunities afforded by the racial mix of the population.

The impending shifts in emphasis also foreshadows a change in the mission of the End Results Section. Up to now the Section has concentrated on research and publications on the survival experience of cancer patients. The introduction of more population-based cancer registries with active programs of cancer epidemiology means that the Branch will focus more sharply on the concept of continuing registration and the exploitation of both epidemiological and patient survival information so generated.

The End Results Section in publication output describing the survival experience of cancer patients continues to satisfy numerous national demands for this type of information. Work has begun on Report No. 4 on "End Results in Cancer" to be distributed at the time of the Seventh National Cancer Conference in 1972. This series of reports has provided the most comprehensive set of data available on the diagnosis and treatment of cancer and the associated survival of patients. For Report No. 4, a completely new, more comprehensive format, taking advantage of the advanced computer capabilities at NIH, has been developed.

The volume of material assembled by the End Results program permit firmer answers to the question: "How many patients are being cured?" One uncertainty in the data arises from the fact that cancer can recur in years after treatment. Long-term survival rates provide a meaningful measure of therapeutic success and the volume of experience at the disposal of the Branch has made possible the computation of 20-year survival rates. The proportion of patients successfully treated turns out to be encouragingly high for those with resectable tumors and with no evidence of spread beyond the site of origin. The 20-year survival rates, adjusted for normal population mortality, are 85 percent for uterus, 75 percent for ovary and 60 percent for colon, rectum, breast and bladder. Another application is made possible by the large volume of data contributed to the End Results Program represented by a study of a rare form of neoplastic disease, lymphomas of extranodal origin. It can be demonstrated that lymphomas originating in the stomach, lungs and tonsils have appreciably better prognosis than carcinomas originating in these organs.

The End Results data can be analyzed to throw some light on questions of host response. For example, Cutler et al. had previously reported that patients with primary breast cancer without microscopic evidence of axillary metastasis, but displaying palpable nodes had higher survival rates than similar patients displaying no palpable nodes. A slide review by Dr. Berg of these cases revealed that the amount of uninvolved node tissue at the apex of the axilla to be a crucial element in favorable prognosis, suggesting that enlarged "benign" nodes at the apex of the axilla may be associated with host immunologic response.

Migrant Studies

The Branch studies of migrant populations oriented to change in site-specific cancer risks, which is supported primarily by research contracts with investigators in the countries of origin, has continued to focus on migrants from Japan, United Kingdom, and Norway to the United States, and on intra-country migration in Colombia. The possibility that cancer in man may reflect an initiator-promoter process has been strengthened by the collective observations. One may hypothesize that "initiator" may involve events occurring very early in a person's life, so that unless the initial stage occurs in early life, cancer will not develop in later life, even though the promoting agent is present. The importance of early exposures varies with site. The recent sharp rise in colon cancer among Japanese migrants to Hawaii suggests that exposures occurring in adult life after arrival in Hawaii have modified the risks for this site.

To illustrate the contributions made by migrant population studies and related inter-population contrasts of risk, we will use the status of work on colon cancer as an example. The major epidemiological clues for colon cancer arise from the suggestive interrelationships among magnitude of incidence rates, localization of tumors and sex, highlighted by the following features:

1. The male excess of colon cancers among Japanese migrants to Hawaii.
2. Male-female ratios close to unity in high-risk populations (types A and A_1)
3. The tendency for low male-female ratios to prevail in low-risk populations (type C_1).
4. A steeper rise in sigmoid/cecum ratios for males than for females as one progresses from low- to high-risk populations.
5. The recent rise in Connecticut of male incidence for cecum and ascending colon to approach the female level of risk.

To account for these findings, the following model has been proposed:

1. In low-risk populations where the disease is "endemic" colon cancers are concentrated in the cecum and female cases are preponderant.
2. When a new etiologic factor is introduced into such a population the transition from an "endemic" to an "epidemic" phase is first expressed as a rise in sigmoid cancers among older men over 55 years.
3. A rise in female sigmoid cancers occurs, but is delayed to a later time.
4. In later stages of an "epidemic", with more intense exposures to the etiologic factor, a rise in cecum and ascending cancers appears. The rise is more marked for males than for females and tends to diminish or obliterate the female excess in cecum cancers prevailing under "endemic" conditions. The changes for cecum and ascending colon are accompanied by similar transitions in the transverse and descending colon, so that the rise in male cases may move as a wave through the colon from the recto-sigmoid junction to the cecum.

The unifying idea in the model is the greater volatility in male response to changed conditions, for which the migrant studies were crucial in directing attention to this phenomenon.

Several migrant population studies have consistently delineated the great difference between stomach and colon cancer in the behavior of the risks in these populations. For stomach, migrants to the United States have consistently displayed risks more characteristic of the country of origin, suggesting a decisive etiological role for events in the first 15 years of life. The rise in colon cancer risks among migrants to approach the prevailing U.S. level suggests that events in adult life play a role, one possibility being that expression of a precursor state may be delayed to older ages. One candidate precursor lesion that would fit this pattern, adenomatous polyps, has been proposed. Adenomatous polyps tend to appear rather late in life and the proportion of polyp bearers under age 35 or 40 is small. This consideration, in turn, has introduced pathology into the study program. Comparative population studies on the congruence of colon cancer and adenomatous polyps have been initiated and such efforts will be extended to the Japanese migrant study. Colons from autopsies in Hawaii and Japan are being assembled in an effort to collate polyp findings with incidence data. The latest development in the migrant study program which is now utilizing the cohort of Japanese males in Hawaii, first assembled by the National Heart and Lung Institute for studies of cardiovascular disease, in studies directed primarily to gastrointestinal cancer at this time. A major investment will be made to relate changes in intestinal flora and diet to the etiology of colon cancer.

The point which emerges with increasing clarity is that the etiology of colon cancer is unlikely to be resolved successfully by studies conducted within the confines of a single population. Only with the broad perspective afforded by comparative population investigations has it been possible to distill ideas on etiology. Colon is probably not unique in this respect and the suspicion is growing that epidemiological findings within any single population will reveal few secrets and that clarification of the epidemiology of many cancer sites will require the broad picture provided by comparative population studies of sedentes and migrants.

Consulting

The Branch extended its consulting activities to many new cancer related problems covering much of the research of the Institute and the impact of cancer on the general population. The staff of the Mathematical Statistics and Applied Mathematics Section have been particularly active with in-house staff research. Work has been done with over a dozen branches and laboratories, ranging from problems in histo-compatability testing to the design and analysis of controlled clinical trials under some very restrictive circumstances. Out of the Office of the Chief has come consultation in problems of epidemiology, carcinogenesis, drug safety evaluation, problems of determining safety levels of radiation, problems of tissue transplantation, and the evaluation of new therapies. There has been extensive cooperation and consultation with the Food and Drug Administration, the National Academy of Sciences, the Atomic Energy Commission, the Federal Aviation Administration, and the Veterans Administration as well as with the World Health Organization, and the International Agency for Research on Cancer. Consultation has also been given to the American College of Surgeons, and the American Cancer Society. Within NIH, but outside the Cancer Institute, consultation has been given to investigators in NHLI, NICHD, NIAMD, and NIDR.

Problems

The dimensions of the problems confronting the Branch set forth in preceding annual reports remain unchanged. The major constraint is the limited number of positions. The Third National Cancer Survey has been slowed down by the tight personnel ceilings and this factor is forcing modifications in survey plans and some curtailment of the ambitious program as originally conceived. We do not have enough consultants on data systems and information processing to meet the demand for services. We are currently unable to entertain development of studies of employee populations greatly desired by investigators concerned with occupational carcinogenesis. In many respects the program of migrant population studies is a holding operation and only a small fraction of the lines of work that have been indicated can be pursued with present staff. The imbalance between available research contract funds and the size of the intramural staff may deteriorate, given larger appropriations for cancer research. The situation can be ameliorated in part by development of the prime contractor concept and a search for prime contractors to operate population-based cancer study centers, which are of interest to the Etiology Area.

The integration of information yielded by clinical trials (a subject for which the Branch provides some supporting staff services) with end-results data provided by cancer registries needs to be improved. Reorganization of the Branch to reestablish a Clinical Biometry Section concerned with not only consultation on conduct of clinical trials but also with review of the end-results data to define topics amenable to further investigation by clinical trials should improve matters.

Despite these caveats the future prospects look good. Mathematical statistical developments, many of them made by Branch staff, promise to yield improved techniques in experimental design and analysis, particularly with respect to problems of carcinogenesis. The migrant studies have opened substantial areas for investigation of hypotheses on cancer etiology. The Third National Cancer Survey will produce important data. The End Results studies have produced promising leads on prognostic factors in breast cancer and useful results may be forthcoming for other sites. Finally, we are now able to identify some members of the Branch staff who will be able to assume greater responsibilities for program direction and replace the present senior staff nearing retirement age.

Honors and Awards

Byar, D.P.: Invited address, Northeastern Section of American Urologic Association, Annual Meeting, September 1970, Hershey, Pennsylvania.

Connor, R.J.: Served on a site visit team for the Clinical Research Centers Grant Committee. Refereed for J. Nat. Cancer Inst.

Cutler, S.J.: General Secretary, International Association of Cancer Registries. Adjunct professor, University of Pittsburgh. Member, NCI Breast Cancer Task Force. Member, Breast Cancer Contract Review Committee. Second National Breast Cancer Conference, Los Angeles, Calif. May 1971. Reviewed for J. Nat. Cancer Inst., Cancer, J. Chronic Dis.

Gart, J.J.: Member, Chemical Carcinogenesis Contract Review Committee. Member, Biometric and Epidemiological Methodology Advisory Committee of FDA. Refereed papers for J. Nat. Cancer Inst., Toxicology and Applied Pharmacology, Biometrics, J. Amer. Statis. Assoc., Technometrics, Science Books: A Quarterly Review. Presented an invited paper at the WHO sponsored Symposium on Quantitative Epidemiology, Moscow, USSR, Nov. 1970. Presented an invited paper at the Seventh International Biometric Conference in Hannover, Germany Aug. 1970. Visiting Scholar, Virginia Polytechnic Institute and State Univ. for 1971. Lecturer, Dept. of Statistics, Pennsylvania State University, Oct. 1970. Member, Regional Advisory Board, Biometric Society. Member, Ad Hoc Review Committee for Biostatistics Fellowships.

Haenszel, W.: Member, American Cancer Society Committee on Epidemiology and Statistics (Dec. 1970). Member, Editorial Advisory Board, Environ. Res. Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health. Traveling Fellow, International Agency for Research on Cancer, World Health Organization. Member, American Association of Cancer Research Committee on Environmental Carcinogenesis. Member, Working Party on Neoplasms for U.S. Subcommittee on 9th Revision of International Classification of Diseases. Invited paper, National Conference on Cancer of the Colon and Rectum, San Diego, Calif. Jan. 1971. Invited paper, Symposium on Studies of Cancer and Other Diseases among Migrants to Israel, Tel Aviv. Feb. 1971.

Kuch, T.D.C.: Reviewer for "Computer Analysis of Labor Progression" paper for publication at request of Dr. Emanuel A. Friedman, Department of Obstetrics and Gynecology, Harvard Medical School, and Mr. Bernard Kroll, Section on Systems Design and Data Processing, Office of Biometry, National Institute of Neurological Diseases and Stroke.

Levin, D.L.: Member, Clinical Trials Contract Review Committee, Chemotherapy, NCI.

Mantel, N.: Invited Speaker, Biometric Society Meetings and the Hannover International Biometrics Conference. Invited Discussant, Washington Statistical Society. Consultant to the Food and Drug Administration, Federal Aviation Administration. Associate Editor, Cancer Research. Editorial Board, Biometrics. Adjunct Professor of Biostatistics, University of Pittsburgh School of Public Health. Reviewer for Ann. Math. Statis; Amer. Statis.: J.Amer. Statis. Assoc.; J.Nat. Cancer Inst.; Technometrics: Cancer; Science and other Journals. Judge in Technometrics Prize Contests. Judge in Washington Statistical Society Prize Contest.

Pettigrew, H.M.: Awarded Ph.D. in Mathematical Statistics from George Washington University, Feb. 1971. Consultant to the Computer and Biomathematical Study Section, Division Research Grants, NIH. Reviewer for Biometrics.

Schneiderman, M.A.: Member, *Ad Hoc* Committee, Advisory Committee to the Federal Radiation Council, Study of Uranium Miners, NAS-NRC. Member, *Ad Hoc* Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens, DHEW. Advisory Panel on Carcinogenicity of Pesticides. Chairman, Animal Data and Human Studies, Subgroup IV, Tobacco Working Group, Lung Cancer Task Force, NCI. Member, Board of Directors, Washington Statistical Society. Member, Advisory Board, Research Support Center, Veterans Administration Hospital, Washington, D. C. Council Member, International Biometric Society. Editor for Invited papers, *Biometrics*. Adjunct Professor of Bio-Statistics, University of Pittsburgh Graduate School of Public Health. Chairman, Regional Advisory Board, Eastern North American Region, Biometrics Society. Clinical Associate Professor, Georgetown University School of Medicine. Reviewer for: *Biometrics*, *Blood*, *Cancer Research*, *JAMA*, *Technometrics* and *Trans. Biomed. Engin.*, *Science*. Member, *Ad Hoc* Committee, Information Retrieval and Ranking of Estimated Risk for Potential Carcinogenic Hazards in Man, NCI.

Weiss, T.: Member of a Contract Review Committee, American College of Surgeons, Chicago, Illinois. Member, Grant Application Review Committee, Special Center of Research, UCLA Medical School, Harbor General Hospital, Torrance, California. Member, Grant Application Review Committee, Special Center of Research, School of Medicine, University of Washington, Seattle, Washington. Member, Contract Review Committee, at the request of Dr. Robert I. Levy, Chief, Lipid Metabolism Branch, NHLI.

Serial No. NCI-4250
1. Biometry Branch, OASDD
Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Activities during FY 1971

Previous Serial Number: Same

Principal Investigators: William Haenszel, Marvin A. Schneiderman, and Nathan Mantel

Other Investigators: David L. Levin, Benjamin E. Carroll, James Koziol, Gary A. Glober

Cooperating Units: Epidemiological Pathology Unit
John W. Berg, Carolyn Lingeman and Henry Feldman

Man Years:	(EPU)	(OC)
Total:	5	6.5
Professional:	3	2.5
Other:	2	9

Project Description

1. General planning and direction of the scientific program of the Branch, including recruitment and development of statistical staff and coordination of activities of statistical units assigned to other Branches of the National Cancer Institute. Mr. Haenszel serves as Chairman of the Biometry and Epidemiology Contract Review Committee. Dr. Schneiderman is a member of the working group on Tobacco and Health.

The major problems of the Branch derive mostly from personnel limitations which have restricted the consulting activities of ADP Management Section, led to delays in data collection and processing for the Third National Cancer Survey, discontinuance of some research projects in Demography, and delayed studies to develop prognostic factors for breast cancer, (undertaken in cooperation with the Health Insurance Plan of Greater New York). A problem repeatedly stressed in previous annual reports -- the need for better regional balance in U.S. coverage for reporting of cancer incidence -- has led to active canvass of the possibilities for setting up additional population-based registers. The straightforward approach would be to transform some of the survey areas being covered in the Third National Survey into continuing registry operations. The most promising areas from the program point of view would be San Francisco-Oakland, Atlanta and Detroit. Any decisions on a new data-gathering apparatus will have to be related to a general, coordinated research program of the National Cancer Institute for problems of etiology in man.

Another pressing problem is the approaching retirement age of many of the senior Branch staff. While active efforts have been made to recruit younger people with promising potential, we have been unable to retain some of our best personnel. The departure of Dr. Bailar to accept a position at Veterans Administration during the past year was a severe loss in this connection. We have the problem in mind and the highest priority is being given to identification and grooming of persons for future section head assignments.

Migrant Studies

The possibility that cancer in man may be a result of an initiator-promoter process has been strengthened by data from the various migrant studies. The "initiator" may involve events occurring very early in the person's life -- so that in the absence of the promoter in later life, cancer will not develop. Thus, the Japanese migrants who have reduced stomach cancer risks are those who have switched over to a Western diet. The importance of exposures in early life vary with site, however. The recent sharp rise in colon cancer among the Japanese migrants suggests that exposures occurring in adult life (after arrival in Hawaii) have changed the risks for this site. The initiator-promoter concept for such hormone related diseases as breast or prostate cancer seems less obvious -- and, in fact, breast cancer among Japanese migrants appears to be the slowest of the major cancers to display the residence-shift effect.

The Migrant Studies Program currently consists of four components: Norwegian, Japanese, Colombian, and Polish. While it is simplest to discuss the progress to date in terms of the separate subprograms, the description of future plans will reflect the integration of the various parts of the program into a coordinated whole.

A. Norwegian Migrant Studies

Through coordination with a Heart and Lung Institute grant to Professor Reid at the London School of Hygiene, and a contract with the University of Minnesota, studies of the U.S.-born children of Norwegian migrants is moving nicely:

1. Histories for a general population sample of 19,000 individuals in Norway have been collected and a roster of 9,200 living sibs of Norwegian-born migrants in the U.S. study sample has been traced and questionnaires collected. A paper on the respiratory symptom data from these samples has been finished and a companion paper for cardiovascular symptoms is in preparation. Five years' follow-up of mortality has been completed.

2. The information on the index migrants in the United States and their living sibs in Norway have been merged and a set of computer tapes containing data on each family of sibships created.

3. In connection with a case-control study of gastrointestinal cancers, a supplementary dietary history from males in the general population and sib samples were collected by mail.

4. The editing and coding of the dietary forms for the general population and sib samples have been completed and the information stored on computer tapes.

5. Case-control interviews for 2121 gastrointestinal cancer patients (stomach, colon, rectum and controls) have been completed and the data have been edited, coded and stored on tape.

6. A pathologist supported by the contract joined staff of the Norwegian Cancer Registry in March 1970 and planning has begun on studies parallel to those previously or currently being carried out in Colombia, Hawaii, Hartford, Connecticut, and several other areas.

B. Japan, Hawaii, and California

Interviewing of stomach cancer patients and controls while completed in Japan, continues in Hawaii, and California with their lesser case loads. Interviewing in all areas continues for colon and rectum cancer, esophageal cancer, and lung cancer. In addition, the California investigator, Dr. Dunn, is developing new protocols for other types of cancer such as breast cancer and has completed interviewing 1000 recent Japanese immigrants to document changes in dietary habits and other epidemiologic variables.

The Pathology program in Sendai, Japan is shifting from concentration on stomach cancer to colon cancer with added analysis of intestinal metaplasia of the stomach. Other work in Sendai has added substantially to our knowledge of the differences in types of lymphoma found in Japan and the U.S. In Nagoya, Professor Akazaki continues his comparative study of latent prostate cancer in Japanese, Colombians, and U.S. residents. Glands are now being collected in New Orleans and Honolulu and Cali.

The Japanese migrant material is being very effectively developed through the exploitation of the Hawaiian material. The separate study component in Hawaii have included or currently include:

1. collection of histories on diet, residence, occupation and other demographic variables from representative samples of the population over 35 years of age;

2. interviews of cases and matched hospital controls for selected cancer sites;

3. completion of pathology protocols, when suitable specimens are available, for patients with cancer of the stomach, esophagus, colon and rectum interviewed in the case-control studies.

During a long visit by Mr. Haenszel to Hawaii, a substantial cooperative effort with NHLI's Honolulu Heart Program was established. Several NCI studies have been begun on HHP special cohort of Japanese men (including a study of cytologic atypias in sputa being undertaken by the Epidemiologic Pathology Unit). Detailed dietary questionnaires are being compared and cross checked, and the HHP clinical chemistry data are being studied in relation to colon tumors.

In collaboration with NHLI a third cycle of examinations of the HHP cohort will begin in late 1971. The NCI protocols for this round of examinations will center on intestinal cancers, the incidence of which is rising rapidly in this population. The specific protocols are now under review and the decisions made will be described in the next annual report.

The parallel pathology programs for the Hawaiian Japanese are well coordinated with other study phases. Dr. Stemmermann has provided a histologic classification of Hawaiian stomach cancer that correlates well with dietary histories on a preliminary analysis and has furnished key documentation to support the hypothesis that colon adenomas are epidemiologically equivalent to colon carcinoma.

C. Colombia

The work in Colombia was materially aided by a 5-month stay (Jan.-May 1970) by Mr. Haenszel and an exchange with Dr. Correa at the time of the International Cancer Congress in Houston. Dr. Correa spent the 1970-71 fiscal year in residence in Bethesda working with Mr. Haenszel on the analysis of the Cali registry and organ bank data.

Recent reports by the Colombian group include further analysis of precancerous changes in stomachs in patients from various parts of Colombia and analysis of the histologic types of stomach cancers seen in Connecticut in foreign-born and native-born individuals from two time periods. This latter project was developed in collaboration with the Epidemiologic Pathology Unit and the Connecticut Cancer Registry. (In related work Dr. Lingeman of the Epidemiologic Pathology Unit has completed a survey of stomach cancer in domestic animals using the same histologic system).

Very high gastric risk areas have been pinpointed in Colombia (the department of Narino close to the Ecuador border). Preliminary surveys and gastric biopsies have shown a high frequency of intestinal metaplasias (a suspect precursor). Further work using a gastrocamera and biopsy to identify cohorts for surveillance classified by gastric mucosa status will be attempted. Analysis of local water supplies suggest the presence of nitrogen compounds, closely related to nitrosamines, and further work on characteristics of local water supplies and soils is underway. Plans for nutritional surveys in selected localities (Pasto, Cartegena, and Cali), were begun. The content of the questionnaires and the specimens to be collected are dependent on the choice of hypotheses indicated by the epidemiological findings on stomach cancer. Implementation will be delayed until better information on the chemistry of the local food crops is obtained. The consultant services of a botanist will be required.

A number of opportunities for prospective studies in the Cali area have been identified, but their utilization depends on local interests and availability of manpower. A recent example of the use of Cali populations was a study of the possible association of herpes virus type II with cervical carcinoma, a leading cause of morbidity and mortality in Cali, undertaken by Dr. Melnick.

D. Poland

Continued accumulation of incidence data by the cancer registries for selected areas of Poland have delineated some of the major features of cancer in that country and these data appear in the second edition of Cancer in Five Continents published by the UICC. Two sites (stomach, breast) show a pronounced urban-rural difference. The rural population has high stomach cancer rates, and low breast cancer rates. Some of this may be a cohort effect -- although the age-specific breast cancer rates strongly resemble those in Japan and Colombia, in contrast to the United States. It is still intended that future PL-480 agreements (after 1972) should be directed toward exploiting comparisons with Poles residing in the United States.

Work on case-control studies for selected sites (stomach, colon, rectum, breast, cervix corpus) has continued. Preliminary analysis of the breast cancer data agree in several respects with the findings of MacMahon and collaborators in other low-risk populations (Japan, Brazil, Greece). The lowest risks are presented by women reporting first pregnancies at a very early age, this effect is most pronounced for breast cancer at pre-menopausal ages.

Arrangements have been made to introduce collection of pathology specimens in Poland for selected sites (thyroid, stomach, colon, rectum). The initial efforts will focus on describing the pathology features of cases in the case-control series and relating them to the other epidemiological findings. Efforts will also be made to improving the histological information on case reports to the registry.

Future Plans

The emphasis has now shifted from data collection and processing to data analysis. We are now at the stage where the data pipeline has been filled and the output of findings can affect the course of future activities. From this point of view the analysis of the case-control studies of gastro-intestinal cancer is signficiant, not only in its own right, but in its impact on the design of studies of other sites.

Pathology activity has been introduced this year in Norway and Poland. We expect Hawaii to maintain its leadership (with Colombia and Sendai) in these coordinated programs. Secondly, we intend to go much deeper into the pathogenesis of the cancers under study, both with our prime migrant investigators and also with the migrant groups as in the proposed study of gut flora.

Under the auspices of the Epidemiologic Pathology Unit, a major effort is being mounted for the study of the role of bacterial flora and diet in the etiology of colon cancer. It is proposed to use the Hawaiian Japanese as a prime resource in this study.

The review of the colon cancer data from several areas now suggests some etiological hypotheses that have been discussed by Haenszel and Correa in a paper presented at the San Diego Colon Cancer Conference and to be published in Cancer. The implications for study of colon will not be reviewed here. We wish merely to make the point that collection of information from several populations yields suggestive patterns that cannot be discerned from studies based on a single population. Much greater effort should be directed to the synthesis of data in hand and for this effort to be successfully carried out, we must enlist greater assistance from the Principal Investigators for our several contracts. We are presently negotiating with Professor Correa (Universidad del Valle, Colombia) with the thought of having him spend two years at NCI as a Visiting Scientist.

Plans to utilize the cohort of Japanese males being followed by the Honolulu Heart Program for cancer studies (with greatest emphasis on gastrointestinal sites) are nearing completion. The Honolulu Heart cohort is an epidemiologically unique group already well characterized and observations on cancer epidemiology can be made on these individuals at a tiny fraction of the cost of creating a special cohort of our own. The intent is also to assemble a cohort of female spouses and to utilize them in studies of breast cancer, as well as for gastrointestinal sites. In addition to studies on intestinal flora, already noted, the cohort studies will permit investigation of suspect etiological factors for other sites. For example, we will be able to relate hormonal profiles for these populations to the subsequence appearance of cancers. For example, clinical prostate cancer shows the migrant effect (rising in Hawaiian-Japanese from the low incidence seen in Japan toward the high U.S. level). Studies of prostatic cancer should command high priority, particularly since it can be coupled with the histological studies of occult prostatic carcinomas now underway in Nagoya.

Gall bladder cancer may be a specifically Japanese disease while liver cancer seems epidemiologically different in Japan and the U.S., and modest pathology studies are proposed in conjunction with the adding of these two cancers to the interview list.

It is proposed to create an organ bank of specimens from the epidemiologically defined Honolulu Heart cohort as a resource for further study.

Pathology studies will be coordinated with studies on similar diseases in domestic and experimental animals and an ever-increasing number of cross-disciplinary and cross-organizational projects within Etiology are being developed.

In all areas, the effort will be to obtain comparative epidemiologic and supporting pathological data while exploiting the special capabilities of each collaborator and the special "experiments of nature" found in the different parts of the world.

Consultation

Dr. Schneiderman has been extensively involved in problems of safety, safety testing, and carcinogenesis. He prepared the Cancer Institute position paper (for use by the National Academy of Sciences) on radiation as a carcinogen. He was a member of the NAS-NRC advisory committee on the radiation exposure of uranium miners. He was part of the planning committee for the NAS-NRC-FDA program for monitoring of adverse effects of drugs, and participated in preparing the recommendations for the establishment of a National Drug Testing Center. Dr. Schneiderman has also been involved in the development of studies of Burkitt's Lymphoma and participated in the epidemiologic planning conference with the IARC at Entebbe, Uganda.

Dr. Schneiderman continues to be the Chairman of the Regional Advisory Board of Eastern North American Region of the Biometric Society. He was elected as a member of the International Council (main governing body) of the Biometric Society. He is Invited Papers Editor of Biometrics, Professor (Adjunct) of Statistics at the University of Pittsburgh Graduate School of Public Health, Associate Professor of Statistics (Clinical) at the Georgetown University Medical School. He remains a member of the Scientific Advisory Board of the Veterans Administration Research Support Center, Washington, D. C. He gave the major invited address at the meeting of the Florida Chapter of the American Statistical Association, "Protecting the Environment by the Numbers."

In the past year Dr. Schneiderman left the Biometry Branch to become Associate Scientific Director for Demography (Acting).

Mr. Mantel has collaborated extensively with investigators in or formerly with the Epidemiology Branch (Glass, Oleinick, Wertelecki, Teitelbaum, Priester, Fraumeni, Burbank, Mulvihill),has assisted in the areas of both cancer chemotherapy and carcinogenesis (Goldin, Weisburger), and has advised other NCI investigators on numerous other problems. He has been consulted by staff of the Food and Drug Administration (Gross) on the problems of drug and food additive safety, of the Atomic Energy Commission (Hollister) on the question of obstetric radiation and childhood cancer, and by other Government officials on traffic and airline safety (Horowitz, Federal Aviation Administration). Miscellaneous assistance has been provided to cancer investigators throughout the country who have had occasion to call on Mr. Mantel. Mr. Mantel's work in technical statistical research during this period has been extensive, including contingency table analysis; variable selection procedures; permutational and non-parametric procedures; competing risk analysis; iterative procedures; tests for dependence; analysis of folded binomial data. His advice and assistance to other investigators doing such statistical research has been widely recognized.

Dr. Levin has provided consultation to several clinical investigators, including a very extensive review of all cases of Mycosis Fungoides treated at the Clinical Center since it opened, and a method of matching patients with prospective transplant donors on the basis of HL-A antigen. He has also worked on several in-house research projects such as determination of low-level environmental carcinogens.

Other activities

The project for evaluating the use of mammography as a screening tool for breast cancer continued to produce encouraging results. The first full follow-up, as reported by Mr. Sam Shapiro of the Health Insurance Plan of Greater New York at the Houston International Cancer Congress, showed 40 percent fewer deaths from breast cancer (31 compared to 52) in the screened group compared to the randomly assigned control group. Mr. Shapiro concluded "Analyses using case fatality rates point to the detection through screening as the factor responsible for this difference."

Honors and Awards

Haenszel, W.: Member, American Cancer Society Committee on Epidemiology and Statistics (Dec. 1970). Member, Editorial Advisory Board, Environ Res. Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health. Traveling Fellow, International Agency for Research on Cancer, World Health Organization. Member, American Association for Cancer Research Committee on Environmental Carcinogenesis. Member, Working Party on Neoplasms for U.S. Subcommittee on 9th Revision of International Classification of Diseases. Invited paper, National Conference on Cancer of the Colon and Rectum, San Diego, Calif. Jan. 1971. Invited paper, Symposium on Studies of Cancer and Other Diseases among Migrants to Israel, Tel Aviv. Feb. 1971.

Levin, D.L.: Member, Clinical Trials Contract Review Committee, Chemotherapy, NCI.

Mantel, N.: Invited Speaker, Biometric Society Meetings and the Hannover International Biometrics Conference. Invited Discussant, Washington Statistical Society. Consultant to the Food and Drug Administration, Federal Aviation Administration. Associate Editor, Cancer Research. Editorial Board, Biometrics. Adjunct Professor of Biostatistics, University of Pittsburgh School of Public Health. Reviewer for Ann. Math. Statis; Amer. Statis.; J. Amer. Statis. Assoc.; J. Nat. Cancer Inst.; Technometrics; Cancer; Science and other Journals. Judge in Technometrics Prize Contests. Judge in Washington Statistical Society Prize Contest.

Schneiderman, M.A.: Member, Ad Hoc Committee, Advisory Committee to the Federal Radiation Council, Study of Uranium Miners, NAS-NRC. Member, Ad Hoc Committee on Evaluation of Low Levels of Environmental Chemical Carcinogens, DHEW. Advisory Panel on Carcinogenicity of Pesticides. Chairman, Animal Data and Human Studies, Subgroup IV, Tobacco Working Group, Lung Cancer Task Force, NCI. Member, Board of Directors, Washington Statistical Society. Member, Advisory Board, Research Support Center, Veterans Administration Hospital, Washington, D. C. Council Member, International Biometric Society. Chairman, Regional Advisory Board, Eastern North American Region, Biometric Society. Editor for Invited papers, Biometrics. Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health. Clinical Associate Professor, Georgetown University School of Medicine. Reviewer for: Biometrics, Blood, Cancer Research, JAMA, Technometrics and Trans. Biomed. Engin., Science. Member, Ad Hoc Committee, Information Retrieval and Ranking of Estimated Risk for Potential Carcinogenic Hazards in Man, NCI.

Publications

DeWys, W., Golden, A., Mantel, N.: Hemotopoietic recovery after large doses of cyclophosphamide: Correlation of proliferative state with sensitivity. Cancer Res. 30: 1692-1697, 1970.

Glass, A.G., Mantel, N., Gunz, F.W., and Spears, G.F.S.: Time-space clustering of childhood leukemia in New Zealand. J. Nat. Cancer Inst., (in press).

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Gross, M.A., Fitzhugh, O.G. and Mantel, N.: Evaluation of safety for food additives: An illustration involving the influence of methylsalicylate on rat reproduction. Biometrics 26: 181-194, 1970.

Haenszel, W.: Studies of migrant populations. Editorial, J.Chron.Dis. 23: 289-291, 1970.

Haenszel, W.: Identification of migrants and their descendents in the United States. J. Chron. Dis. 23: 383-387, 1970.

Haenszel, W. and Correa, P.: Cancer of the colon and rectum and adenomatous polyps: A review of epidemiological findings. Cancer, (in press).

Johnson, R.E., Thomas, L.B., Schneiderman, M.A., Glenn, D.W., Faw, Frederick, Hafeman, M.D.: Preliminary experience with total nodal irradiation in Hodgkin's disease. Radiology, 96: 603-608, 1970.

Mantel, N.: Why stepdown procedures in variable selection. Technometrics, 12: 621-625, 1970.

Mantel, N.: Letter, More on variable selection and an alternative approach. Technometrics (in press).

Mantel, N.: Letter, Supplements to the Elston-Stewart test of association. Biometrics, (in press).

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Mantel, N.: Letter to the Editor. Amer.Statis, 24: 50, 1970.

Mantel, N.: An alternative test for the symmetric folded binomial distribution. Biometrics, 26: 848-851, 1970.

Mantel, N. and Bailar, J.C.,III: Letter, Model I or Model II in competing risk analysis? Biometrics, 26: 861-863, 1970.

Mantel, N. and Bailar, J.C.,III: A class of permutational and multinomial tests arising in epidemiological research. Biometrics, 26: 687-700, 1970.

Mantel, N. and Myers, M.: Problems of convergence of maximum likelihood iterative procedures in multiparameter situations. J. Amer. Statis. Assoc., (in press).

Schneiderman, M.A. and Scotto, Joseph: Computer-investigator interactions in small scale clinical trials. In the Proceedings of the Fifth Scientific Meeting of the International Association, Primosten, Yugoslavia, August 24-31, 1968, pp. 449-467.

Schneiderman, M.A.: Letter on screening procedures. Cancer Chemotherapy Reports (Part 1) 54: 5, 293-301, 1970.

Teitelbaum, M.S. and Mantel, N.: Socioeconomic factors and sex ratios at birth. J. Biosocial Science, (in press).

Teitelbaum, M.S., Mantel, N., and Stark, C.R.: Limited dependence of the human sex ratio upon birth order and parental ages. Amer J. Hum Genet., (in press).

Serial No. NCI-4250

Zipkin, I., Baer, P.N., Hawkins, G.R., Zucas, S.M. and Mantel, N.: The effect of fluoride and dietary protein levels on calculus formation, alveolar bone loss, selected salivary constituents and fluoride deposition in the bones and teeth. J. Periodont, 41: 430-437, 1970.

Serial No. NCI-4254
1. Biometry Branch, OASDD, Etiology
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Cancer in a Defined Population (Connecticut)

Previous Serial Number: Same

Principal Investigator: Roger Connelly

Other Investigators: Sidney J. Cutler, William I. Lourie, Jr., Herman Heise, Lillian Axtell, Max Myers

Cooperating Units: Connecticut State Department of Health

Man Years:
Total: 4
Professional: 2
Other: 2

Project Description

Objectives:

To provide data on cancer incidence, methods of treatment, and end results; to investigate the effect on incidence of factors such as urban environment, nativity, economic status, occupation, diet, and personal habits; and to study in detail the interrelationship between the condition of the patient at diagnosis, the therapeutic course, and the end results, in the general practice of medicine.

Methods Employed:

Hospitals accounting for all but 5 percent of the approved general hospital beds in the State participate in a case reporting and follow-up program administered by the Connecticut Health Department. Samples of cases reported to the registry are drawn for special study involving the examination of histologic specimens, and the collection of histories from patients and relatives.

Major Findings:

During the current year major emphasis was placed on reducing the time lag in the reporting of cases and on the preparation of data on individual patients for use in End Results Report No. 4 to be published in the Spring of 1972.

Significance to the Program of the Institute:

The Connecticut Cancer Registry is the most extensive population-based program in the United States which provides reliable information on cancer incidence and mortality and information on the end-results of treatment. The completeness of case reporting and the efficiency of patient follow-up make possible the evaluation of the impact of case findings and treatment programs on the cancer problem as it affects a defined population. It provides a continuous resource for the study of cancer epidemiology and of the influence of social and biological factors on the end-results of treatment. It also provides a basis for evaluating the larger body of data obtained via the End Results Program.

Proposed Course of Project:

1. Roger Connelly, on detail from the Biometry Branch to the Connecticut Tumor Registry, is conducting an extensive record linkage study to test the hypothesis that there is an association between infectious mononucleosis and the risk of developing hematopoietic neoplasm. In addition to the substantive findings that will be forthcoming, this study will provide useful experience in record linkage methodology in the investigation of cancer etiology. The analysis of the findings will be completed by Summer 1971.

2. A review of the sampling of histologic specimens of cancers of the bladder from different calendar periods is now in progress. The purpose is to determine whether the reported increase in the incidence of bladder cancer may be due to a shift in diagnostic criteria.

3. We aim to develop an active collaborative working arrangement between the Connecticut Tumor Registry and one of the universities in the State. The primary goal is to develop an investigational team that will be able to fully exploit the research potential of the registry. Two operational elements are required: (1) A competitive salary scale for the full time key staff members (which can not be established through the Health Department), and (2) access to a variety of medical specialists and other biomedical investigators who are needed on an *ad hoc* basis to plan and carry out specific studies.

Serial No. NCI-4257
1. Biometry Branch, OASDD, Etiology
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Collaborative Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigators: Sidney J. Cutler, William Haenszel

Other Investigators: William I. Lourie, Jr., Lillian M. Axtell, Herman W. Heise, Roger R. Connelly, Max H. Myers, John W. Berg, Ardyce Asire, Benjamin Carroll, Bruce Hyman, Evelyn Shambaugh, Benjamin Hankey

Cooperating Units: California Tumor Registry; Connecticut Tumor Registry; Massachusetts Cancer Register; Charity Hospital of Louisiana; Indiana University Medical Center; New York Medical College; State University of Iowa Hospital; University of California Medical Center; University of Chicago Clinics; University of Michigan Hospital; University of Virginia Hospital; Norwegian Cancer Register; Unit on Epidemiologic Pathology, Tohoku University; University of Hawaii; California Department of Public Health

Man Years:	
Total:	14
Professional:	9
Other:	5

Project Description

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs for the collection and analysis of data on cancer in human populations and on the end results of treatment. This includes consultation and advice on methodological problems, development of improved statistical tools for the analysis of observations on human populations, and active collaboration in the collection and analysis of data in specific projects.

Methods Employed:

1. The consultation draws on standard statistical and actuarial techniques and accepted principles for records management, with necessary modifications required for any individual study situation. Many of the problems can be handled by correspondence, but field trips are frequently required. Consideration must be given to the study objectives of the individual investigator or agency, the existing administrative arrangements, and the personnel and financial means at hand to implement the program of operations and studies.

Most of the consultation work in this area concerns problems in the integrated use of data from various study techniques: analyses of mortality data, controlled retrospective and prospective studies of selected cohorts, morbidity surveys, screening of populations for precursive signs, and cancer registries. The Branch staff is particularly interested in the methodological problems and issues involved and will continue to do research in this area.

2. Provide staff support to the End Results Group, which represents more than 100 hospitals participating in a cooperative program for the evaluation of the end results of cancer treatment. This includes advice on the development and applications of uniform definitions and procedures for participating cancer registries, consultation on planning and implementing special studies, editing and analysis of data submitted to the National Cancer Institute in accordance with a Uniform Punch Card Code, and exploration of more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data. In addition, leadership is provided in establishing working relationships with cancer registry programs in other countries.

The End Results Evaluation Program provides a baseline for planning and for interpreting controlled therapeutic trials, and for evaluating results reported by specialty centers.

The resources of the End Results Group provide a continuing opportunity for evaluating and testing the recommendations of the American Joint Committee on Staging the End Results Reporting. Liaison with this committee is facilitated by the fact that Dr. Cutler is a member of this committee and regularly attends committee meetings.

The data collected through the End Results Group (currently 40,000 new cases per year) provide a basis for evaluating trends in patient characteristics, stage of disease, treatment practices and associated end results in the general run of patients. Major contributions are made by Connecticut, which is a population-based registry, Alameda County, California which is the population-based component of the California Tumor Registry and by Massachusetts, which is developing a population-based registry in the Boston area. The population-based registries make possible evaluation of trends in patient survival in the light of trends in cancer incidence. The wide variety of institutions participating in the program make possible comparison of experience in university and non-university hospitals, government supported (county and city) and privately supported hospitals, large and small hospitals. In addition, this broad data resource makes possible the selection of specific series of cases for detailed study through review of hospital records. Of particular advantage is the availability of sufficiently large numbers of cases for study of relatively rare forms of cancer.

Collaboration with cancer registries in other countries is facilitated in part through the newly formed International Association of Cancer Registries. Dr. Cutler is the General Secretary.

Major Findings:

1. A firm answer to the question: "How many patients are being cured?" is unavailable because cancer can recur many years after treatment. However, a long term survival rate provides a meaningful measure of therapeutic success. The 20-year rate, adjusted for normal mortality expectation, is such a measure. Available data on patients treated since 1940 permit projection of 20-year adjusted survival rates for patients treated during the 1960's. The estimated proportion of successfully treated patients (20-year adjusted survival rate) is encouragingly high for those with resectable tumors and no evidence of spread beyond the site of origin, e.g., 85 percent for uterus, 75 percent for ovary, 60 percent for colon, rectum, breast and bladder.

2. The clarification of criteria for precancerous lesions of the breast is of interest both for purposes of helping identify high risk women and for elucidating the natural history of breast cancer. A system for grading atypia in the breast has been developed by Black and Chabon of the New York Medical College. Application of this scheme to a series of cases in Saskatchewan, Canada reveals that this grading system does identify women who are at high risk of developing cancer. The original biopsy specimens were reviewed "blind" and divided into two categories: (1) significant atypia (grades 3 or 4), and (2) no significant atypia (grades <3). The first category (grades 3 or 4) had a 5-fold relative risk of subsequently developing cancer in comparison with the second (grades <3).

3. Last year Cutler, Asire, and Taylor reported that women who were still menstruating at diagnosis of disseminated recurrence of cancer of the breast had a more favorable prognosis than postmenopausal women. This observation led to an inquiry concerning the prognostic significance of menopausal status at diagnosis of primary breast cancer, with emphasis on probability of escaping disease recurrence. It appears that artificial menopause prior to diagnosis of the primary is associated with favorable prognosis. This advantage is maintained even after adjustment is made for other relevant factors, e.g., age, tumor size, node involvement. The biologic significance of this finding warrants further study.

4. The extensive data bank provided by the End Results Program made possible a study of lymphomas of extranodal origin--a rare form of neoplastic disease. Patients with lymphomas originating in the stomach, lungs, or tonsils were found to have an appreciably better prognosis than patients with carcinomas originating in these organs.

5. Cutler et al. previously reported that among patients with primary breast cancer and no microscopic evidence of axillary metastases those with palpable nodes considered not to be suggestive of metastases had a higher survival rate than patients with no palpable nodes. John Berg (Epidemiologic Pathology Unit) has reviewed the slides on these cases in a search for clues to explain the unexpectedly good prognosis of patients with palpable but non-suspicious nodes. He has found that the amount of uninvolved node tissue at the apex of the axilla appears to be a crucial favorable factor. This suggests that enlarged "benign" nodes at the apex of the axilla are associated with host immunologic response.

Significance to the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigations and cancer registries. An example of the interaction between epidemiologic and end-results investigations are the developing studies of prognostic factors and the role of these factors in cancer etiology.

Proposed Course of the Project:

1. A reorientation of the End Results Evaluation Program, placing greater emphasis on population-based registries, has been started. The California Tumor Registry is being reorganized in order to provide complete population coverage in five counties around San Francisco. Massachusetts is making good progress in developing a population-based registry in the Boston area. The possibility of utilizing the Third National Cancer Survey as a base for developing one or two additional population-based registries is being actively pursued. The goal is to develop several broad based research resources for a wide variety of studies.

2. We will continue to work with the American Joint Committee on Cancer Staging and are now actively analyzing a very large melanoma series.

3. Effective working liaison with Memorial Hospital (N.Y.C.) and with University of California School of Medicine in San Francisco is continuing. Possibilities for developing working relationships with other cancer specialty centers are being explored. Active involvement in a specific study is a good way to begin. We are currently collaborating with the Oncology Research Center in Madison, Wisconsin in a study of a large series of breast cancer patients treated with 5FU.

Publications

Cutler, S.J., Asire, A.J., and Taylor, S.G., III: An evaluation of ovarian status as a prognostic factor in disseminated cancer of the breast. Cancer 26:938-943, 1970.

Cutler, S.J., Axtell, L.M., and Schottenfeld, D: Clinical assessment of lymph nodes in carcinoma of the breast. Surg. Gynec. Obstet. 131:41-52, 1970.

Lipworth, L., Abelin, T., and Connelly, R.R.: Socioeconomic status and cancer survival. J. Chron. Dis. (in press)

Shedd, D.P., von Essen, C.F., Connelly, R.R., and Eisenberg, H.: Lip cancer in Connecticut. Ca. (in press)

Serial No. NCI-4258
1. Biometry Branch, OASDD, Etiology
2. Demography Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Staff Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigator: Sidney J. Cutler

Other Investigators: Haitung King, Joseph Scotto

Cooperating Units: University of California Medical School
New England Deaconess Hospital
Lahey Clinic
Connecticut Tumor Registry

Man Years:

Total:	3.5
Professional:	1.5
Other:	2.0

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisons between high- and low-risk populations; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagnosis, or successful treatment of cancer; and to present the results in an intelligible and usable form to medical students and practicing physicians as well as to other research workers.

Methods Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

Major Findings:

1. There are no noteworthy findings to be reported. No ad hoc studies are being actively pursued now because of the demands of the Third National

Cancer Survey. Also, some of the studies being conducted by Dr. Bailar have been taken with him to the Veterans Administration.

2. Regular surveillance of cancer incidence and mortality rates, both here and abroad, has been continued. A detailed study of cancer mortality in 1959-61, supplementing and extending the report by Gordon, Crittenden, and Haenszel (NCI Monograph No. 6) has been completed but publication is not contemplated until we can add the mortality data for 1964-1966 and subsequent years.

3. Staff have been active in advisory and consultative services. These include refereeing and reviewing papers for the Journal of the National Cancer Institute, Cancer Research, and many other journals, consultation to outside committees, etc.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, measures trends in cancer rates which may reflect the action of various etiologic agents, identifies high- and low-risk population groups which can be studied and compared, indicates areas for intensive efforts at case finding and follow-up, and provides for the correlation of laboratory studies of cancer with observations on human populations. *Ad hoc* investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

Work under this and related projects provides a tie between epidemiologic and survival studies of cancer. The use in epidemiologic investigations of data originally collected for survival studies (prostatic and bladder cancer surveys) and the reverse (Connecticut-Southwest Region cervical cancer study) are both leading to interesting and potentially significant results with much less cost in time and effort than if separate studies had been undertaken.

The projects now in progress or recently completed reflect the particular interests of staff members. Substantial progress has been made in several areas but a vast array of other important problems will remain untouched until interested and qualified investigators are available. This is especially true since there has been a very heavy drain of Demography Section personnel to work on the Third National Cancer Survey (see NCI-4268). Much of the work reported in the past reflected the personal interests of Dr. Bailar, who left to become Director of Research for the Veterans Administration. The direction of future work will depend greatly on the personal attributes of replacement(s) for Dr. Bailar.

Proposed Course of the Project:

The immediate course of this project can not be predicted because many *ad hoc* studies are undertaken on short notice, as their need becomes apparent and as staff time is available. A number of studies are in various phases of completion, but the items listed below represent long-range goals and shortage of staff resources will not permit their completion and publication in the coming year.

1. The series of reviews of the literature on various aspects of cancer epidemiology will continue. In the coming year reviews of cancer incidence and mortality in the Soviet Union, and of esophageal cancer will require additional documentation. Efforts in this direction will be limited to staff support for Dr. Bailar, who continues to direct this work from his Veterans Administration office.

2. Assembly of data for revision of Cancer Rates and Risks, a short book for practicing physicians, medical students, and others, continues. This book has attained wide distribution since its publication in 1964, and we anticipate publication of another edition after the incidence data from the Third National Survey becomes available. The target date for the new edition is 1974.

3. Hospital records of approximately 800 patients with multiple myeloma have been abstracted and the data are being analyzed. This will be the first such study based on defined population, and should answer many questions about the epidemiology and survival patterns of the disease.

4. The follow-up of mortality for defined cohorts of clergymen will be continued. Analysis to date demonstrates a reduced risk of death in all age groups and for most major causes of death, including many cancer sites, but the reasons for these reduced risks are not yet known.

5. Approximately 1000 cases of cancer of the prostate and urinary bladder and matched controls have been interviewed and the records coded, and analysis is well underway. Computer printouts have been obtained for two case study groups and three control groups. The data are on punch cards and most of the editing has been done. The analysis will be structured to first compare the collected information which has been already keypunched under the following categories: demographical information (i.e., race, place of birth, national origin, residence history, etc.); medical information (i.e., symptoms, treatment, related diseases, family history of cancer, cause of parents' death); social information (i.e., religious affiliation, marital history, lifetime sex activity, educational level, use of tobacco); dietal information (i.e., beverage usage, use of select foods and methods of preparation); occupational information (i.e., employment history, occupationally related exposures to carcinogens). Further analyses will develop from this and it may be necessary to recode more informational details from the original interview schedules.

Honors and Awards

King, Haitung: Clinical Professor of Community Medicine and International Health, Georgetown University Medical School. Research Associate, Center for Population Research, Georgetown University. Advisor-Consultant, D. C. Interagency Council on Smoking. Reviewer for JNCI.

Serial No. NCI-4258

Publications

Bailar, J.C. III, Eisenberg, H. and Mantel, N.: Time between pairs of leukemia cases. Cancer 25:1301-1303, 1970.

King, H.: What we know and don't know about the health of clergymen. In Nix, J.T. and Fecher, C.J. (Eds.): Stamina of the Apostolate, Washington, D.C. CARA, Information Service, Center for Applied Research in Apostolate, 1970, pp. 98-116.

King, H.: Health in medical and other learned professions. J. Chron. Dis. 23:257-281, 1970.

Bailar, J.C. III and Byar, D.P.: Estrogen treatment for cancer of the prostate: Early results with 3 doses of Diethylstilbestrol and placebo. Cancer 26: 257-261, 1970.

Serial No. NCI-4260
1. Biometry Branch, OASDD, Etiology
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Statistical Consultation for the Veterans Administration Urological Research Group

Previous Serial Number: Same

Principal Investigator: David P. Byar

Other Investigator: Harold Newball

Cooperating Units: V.A. Urological Research Group (20 hospitals)

Man Years:

Total:	3.5
Professional:	2
Other:	1.5

Project Description

Objectives:

To provide consultative services in statistical and epidemiological methodology necessary for the various programs sponsored by the Veterans Administration Urological Research Group. This includes assistance in the design, interpretation and evaluation of the clinical trials conducted by the Veterans Administration Urological Research Group, and provision for studies operating concurrently with the clinical trials such as epidemiological studies.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New techniques are developed by the personnel working with the Section to handle specific problems.

Major Findings:

This Section assists the investigators in developing and evaluating methods of treatment for cancers of the male genitourinary tract. Such assistance is generally acknowledged in the publication of findings of the medical investigators. New statistical techniques are published independently.

The Section is more deeply involved in five separate clinical trials:

(a) Main Prostate I, which compares the effect of two treatments (prostatectomy alone, and prostatectomy with estrogen) for Stages I-II, and of four treatments (estrogen, orchiectomy, estrogen plus orchiectomy, and placebo control) for Stages III-IV. The intake of new patients into this study was terminated on March 31, 1967. A total of 2,315 patients had been entered by that date.

(b) Main Prostate II, which also compares the effect of two treatments (prostatectomy alone and placebo control) for Stages I-II and of four treatments (three levels of estrogen and placebo control) for Stages III-IV. This study is designed as a sequel to Main Prostate I. Intake of new patients was terminated in June 1969 after 561 patients had been entered in the study.

(c) Main Prostate III, which compares placebo vs. prostatectomy for operable Stage I-II patients. Inoperable Stage I-II patients are randomly assigned to placebo or 1.0 mg. DES. All eligible Stage III-IV patients are randomized to one of four treatments: 1.0 mg DES, 30 mg Provera, 2.5 mg. Premarin, or 30 mg Provera plus 2.5 mg Premarin. As of October 20, 1970, 328 patients had been entered in this study.

(d) Focal Carcinoma, which compares several treatments for very early prostatic lesions of the type commonly found in TUR or autopsy specimens from old men. There are now 182 patients in this study.

(e) Urinary Bladder, which compares surgery alone, radiation alone, and combined therapy for bladder cancer. There have been 72 patients admitted.

In addition to these five clinical trials, the VA group has several other studies for which NCI provides consultation as requested.

The data from three trials -- Main Prostate III, Focal Carcinoma, and Urinary Bladder -- are not yet sufficient for detailed study.

The results of Main Prostate I have already caused widespread changes in the general treatment of prostatic cancer, because of the finding that the standard estrogen treatment for prostatic cancer carries a very great risk of death from cardiovascular diseases. In addition, the clinical benefits with respect to the cancer are smaller than was generally believed. Overall mortality rates in Stages I, II, and III, and in "early" Stage IV are substantially higher in estrogen-treated patients than in those not receiving estrogens. This hazard was not recognized before the results of this study were available.

During the past year, efforts in this project have been concentrated on three objectives: elucidation of the mechanisms by which estrogen exerts its harmful effects, classification of patients with respect to the probable benefits or losses associated with each treatment tested, and studies (Prostate II) on the effects of various doses of diethylstilbestrol.

Our efforts at elucidation of mechanisms continue to meet great frustration. At the time that Main Prostate I was organized there was no reason to suspect an adverse effect of estrogen on the cardiovascular system, so much important information was not collected and submitted in a uniform way. We are now attempting to gather some items retrospectively (pre-treatment EKG, history of heart disease, admission blood pressure, etc.) but the analysis will be difficult. Main Prostate II includes very careful study of the cardiovascular system before treatment and at each follow-up, and may eventually provide answers to this problem.

Classification really involves two problems--prediction of the (good) effects of treatment on the cancer, and prediction of the (bad) effects on the cardiovascular system. We now have a simple and very accurate way to measure the probability of death from cancer within the first three years after treatment, and this method can be extended to cover later intervals when we have more long-term follow-up data. Prediction of bad effects has been much more difficult, and we do not yet have a good system for identifying a group of patients who are likely to have an adverse reaction to estrogen.

Preliminary results from Main Prostate II indicate (1) that there is no evidence thus far to show that 5.0 mg DES is superior to 1.0 mg in controlling the cancer in either Stage III or IV, (2) that in Stage III there are substantially fewer heart and cardiovascular deaths on 1.0 mg. DES compared to the 5.0 mg dose, (3) that in Stage IV both the 1.0 mg and 5.0 mg doses are more effective than the 0.2 mg dose in controlling the cancer, and (4) that in Stage III the superiority of placebo over the 5.0 mg dose (and to a lesser extent over the other two doses of DES) is accounted for by the excess deaths from non-cancer causes in the estrogen treated patients. Taking all these rather complex relationships into account we see no justification for the continued use of 5.0 mg DES in either Stage III or IV.

It must be noted that cardiologists have not accepted the estrogen findings as readily as urologists. Results of the VA study have been of concern to The National Heart and Lung Institute because of its involvement in the Coronary Drug Project in which several drugs (including estrogen) are actually being used in the hope of reducing mortality among men with a recent myocardial infarct. NHLI assigned a staff member to make a detailed review of our original records and other data on Stages I-II, where the adverse effect of estrogen is most marked. His review completely supported all of our published conclusions. In November 1970 the first report of the Coronary Drug Project appeared (JAMA 214: 1303, 1970). In this report data were presented which indicated that 5.0 mg conjugated estrogen daily led to a significant increase in non-fatal events of myocardial infarction, pulmonary emboli, and thrombophlebitis compared to the placebo group. This treatment has since been dropped from their study.

Significance to the Program of the Institute:

This Section serves the members of the Veterans Administration Urological Research Group by assisting in preparing and revising protocols, forms, and records; giving statistical guidance in the design and execution of clinical trials; processing and analyzing data that is received daily on patients in the various studies; and assisting the members of the Group with reports on the progress of the various studies.

In return for these services, the Demography Section has access to the largest complete set of data available anywhere on the course of prostatic cancer. Over 2,300 patients have been entered in Main Prostate I with follow-up as long as ten years. These data are proving extremely useful and interesting not only in the comparison of treatment effects, but also in defining the general behavior of prostatic cancer in relation to a host of personal, clinical, and laboratory characteristics.

Proposed Course of the Project:

The cooperative studies undertaken jointly by the Veterans Administration and this Section are planned to be a self-generating sequential series to continue as long as fifteen years. Consultative services of this Section have expanded considerably over the past year as the data from Main Prostate I have ripened and as patients have accumulated in Main Prostate II.

In the coming year, we will continue to give most of our attention to the analysis of Main Prostate I and II, and especially of the patients in Stages III-IV, since the other studies do not yet include large numbers of patients with long-term follow-up. In particular, we plan to continue a detailed histological classification for as many patients as possible with frequent study of all data at hand.

Studies of specific cardiovascular effects of estrogen on patients in Main Prostate I have been undertaken, but the data are scanty and difficult to interpret. Main Prostate II was designed to include detailed heart studies, but it will take several more years to collect sufficient data for a full analysis.

Because the amount of useful data collected in these studies is more than our present staff can adequately analyze, contracts for assistance in the analysis were offered to the Yale University Department of Epidemiology and Public Health. Four specific projects are included in this contract: (1) further work on an exponential survival model (multivariate) already begun by us in the past three years (2) a detailed study of the effects of change of treatment during the study (3) a descriptive study of the natural history of untreated cancer of the prostate (based on the placebo treated patients), and (4) a detailed study of the biochemical data collected on all patients in study II in an effort to find measurements which might predict in advance which patients will suffer adverse effects from estrogen treatment. This contract (70-2287) was accepted and funded last year and active work was begun in September 1970. Since that time intensive work on the first project (further development of an exponential survival model) has been carried out.

Honors and Awards

Byar, D.P.: Invited address, Northeastern Section of American Urologic Association, Annual Meeting, Sept. 22, 1970, Hershey, Pennsylvania.

Publications

Bailar, J.C., III and Byar, D.P.: Estrogen treatment for cancer of the prostate: Early results with 3 doses of Diethylstilbestrol and placebo. Cancer 26: 257-261, 1970.

Blackard, C.E., Doe, R.P., Mellinger, G.T. and Byar, D.P.: Incidence of cardiovascular disease and death in patients receiving Diethylstilbestrol for carcinoma of the prostate. Cancer 26: 249-256, 1970.

The Veterans Administration Co-operative Urological Research Group: Carcinoma of the Prostate: Results of the Veterans Administration Clinical Trials. Minnesota Medicine (in press).

Bailar, J.C., III and Byar, D.P.: Expectant treatment of early inoperable (Stage III) carcinoma of the prostate. Current Controversies in Urologic Management, W.B. Saunders Co. (in press).

Serial No. NCI-4265
1. Biometry Branch, OASDD, Etiology
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Consulting in Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew, Robert J. Connor

Other Investigators: Jun-mo Nam, Donald G. Thomas, Alroy M. Smith

Cooperating Units: None

Man Years:
Total: 4.0
Professional: 3
Other: 1

Project Description

To provide consulting assistance and collaboration to NCI scientists on the applications of mathematical statistics and applied mathematics to the interpretation and analysis of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research, (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators, and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program.

Methods Employed:

The methodology of applied mathematics, mathematical statistics and probability are applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem. The members of this section consult with a great many investigators in all areas of intramural research in the NCI.

Major Findings:

During this year the staff consulted with over a dozen different branches and laboratories of the NCI sometimes with several investigators in a single branch or laboratory. In addition the staff has consulted with other researchers at other Institutes of NIH.

Hugh M. Pettigrew has consulted with various members of the Surgery Branch during the past year. One of his major collaborations has been with Miss Hilda Wexler and Dr. Paul Chretien in the design and analysis of a series of experiments to study various aspects of the metastatic behavior of animal tumor systems, particularly the problem of the detection of circulating tumor cells in the blood, and the relationship of host immunity to metastatic behavior. Other members of the Surgery Branch with whom he has consulted are Drs. Pilch, Ramming, Solowey and Sample.

Dr. Pettigrew has also been serving as the statistical consultant for a clinical trial conducted by the Leukemia Service to investigate the value of the Life Island in the treatment of acute myelogenous and acute lymphocytic leukemia. He is now in the process of analyzing data obtained by Dr. Alan Schreiber from the records of the patients who have entered the study up until the present time.

Dr. Pettigrew has also consulted with scientists in the Viral Oncology area. For the Viral Leukemia and Lymphoma Branch, he has analyzed data obtained by Dr. John Pearson in a study to determine the effects of certain clinically active drugs on a guinea pig leukemia, and has also consulted on a variety of topics with Dr. Willie Turner. He has consulted with Dr. Arnold Fowler on the analysis of data from an experiment to determine the effects of oxygen exposure on EDH and SDH levels in mice.

He has consulted with Drs. John Glynn and Arthur Goldberg of the Drug Evaluation Branch on methods to analyze data from an experimental design to screen for immunosuppressors, and, with Donald Thomas, has constructed a computer program that is capable of rapidly processing such data. Robert J. Connor joined the section during the year from the Atomic Bomb Casualty Commission in Japan. Dr. Connor has already entered into consultation with several researchers around the NCI. He is advising Dr. Zbar of the Biology Branch on the appropriate experimental design and tests for comparing treatment of tumors induced in mice. Dr. Connor discussed with Dr. Rodbard of the Endocrinology Branch statistical procedures to be used in a bioassay analysis. He advised Dr. Wunderlich of the Immunology Branch on a problem comparing the effectiveness of normal and diseased cells in destroying target cells. Among the other researchers who have collaborated with Dr. Connor are Dr. W. Strober of the Metabolism Branch, Dr. Bartlett of the Biology Branch, and Dr. Virginia Evans of the Laboratory of Biology.

John J. Gart has collaborated with Dr. Whang-Peng of the Human Tumor Cell Biology Branch on studies of the cell cycle characteristics of blast cells from patients with chronic myelocytic leukemia. He is co-author of a paper on the subject submitted for publication. Dr. Gart has worked extensively with Jay Robbins of the Dermatology Branch on the analysis of grain count data in the disease Pigmentosa Xeroderma as well as on other problems in calibration with counting data. Dr. Gart has advised Dr. Bates of the Experimental Pathology Branch on the proper analysis of animal chemical carcinogenesis problems, particularly with the testing of pesticides for their possible harmful effects. Dr. Gart has consulted with Dr. DiPaolo of the Biology Branch on problems in the quantitative assay of chemical carcinogens. Among his other consultees in the past year on various statistical problems have been Dr. Gail of the Viral Biology Branch, Dr. M. Stanton of the Laboratory of Pathology, Dr. Rodbard of the Endocrinology Branch and Dr. Wunderlich of the Immunology Branch.

Jun-mo Nam collaborated with Dr. Herberman of the Immunology Branch on a large project evaluating the effect of disease (particularly Burkitt's lymphoma, infectious mononucleosis, and leukemia) on the natural cytotoxic antibody in human serum. Mr. Nam is co-author of a paper on this subject submitted for publication in Journal of the National Cancer Institute. Mr. Nam has also consulted with Dr. Yun of the laboratory of Pathology on the study of the effectiveness of enzyme treatment on various tumors in mice.

Before leaving the Section during the year to join the Faculty at Montana State University, Martin A. Hamilton consulted with several researchers. Dr. Hamilton analyzed data of Dr. Broder of the Chemotherapy Evaluation Branch with a view toward ranking the clinical effectiveness of certain drugs. He consulted with Dr. Polmer of the Immunology Branch on the analysis of immunoglobulin measurements in immunodeficient patients and normal control. He advised Dr. Yankee of the Medicine Branch and Dr. Lundberg of the American Red Cross Blood Research Laboratory on the efficacy of fresh to frozen blood in maintaining platelets by transfusion in thrombocytopenic patients.

Donald G. Thomas served as the Section's expert on high speed computing often consulting with other members of the section and branch on the many projects which depend on the use of computers. He has continually evaluated the available computer systems both in house and outside so that the section may utilize those best suited to its needs. Some of Mr. Thomas's computer algorithms reported in the research project are frequently used in the consulting reported above.

Alroy M. Smith plays an important supportive role in the data analysis of the section. In particular in the past year she has written a complex computer program which implements the method devised by Dr. Gart for finding approximate confidence limits for the common odds ratio (or relative risk) from several 2 x 2 tables. Mrs. Smith has used this method along with others to analyze more intensively, under Dr. Gart's direction, the Bionetics Report on tumorigenicity of several pesticides.

Members of the Section have also collaborated with various researchers outside the NCI. Dr. Connor consulted with Dr. G.T. Ross of the NICHHD on a study relating FSH and LH hormone levels with breast cancer. Dr. Connor also consulted with Dr. Sober of NIAMD on planning a study of assay methods in relation to cystic fibrosis. He also reviewed two papers for the Human Genetics Branch of the NIDR. Dr. Connor collaborated with former colleagues at the ABCC on the radiation effects on survivors at Hiroshima and Nagasaki. He is the co-author of one paper submitted to the Annals of Internal Medicine and also co-author of an ABCC Technical Report. Mr. Nam consulted with Dr. Kulin of the NICHD on the analysis of data on the suppression of urine FSH by Ethinyl Estradiol. Dr. Gart and Mrs. Smith analyzed data of Mr. Ederer of the NHLI on the relative risks in the Diet-Heart Study.

Significance of the Problem to the Institute:

Members of this Section are assuming an essential role in much research within the NCI. Their activities include not only statistical analysis but also in planning valid experiments.

Proposed Course of the Projects:

Several of the projects mentioned in the Major Findings section will continue. In particular the consultation with the chemical carcinogenesis area is expected to continue.

Honors and Awards

Dr. Connor served on a site visit team for the Clinical Research Centers Grant Committee. Dr. Gart continued to serve on the Chemical Carcinogenesis Contract Review Committee. Dr. Gart was appointed a member of the Biometric and Epidemiological Methodology Advisory Committee of the FDA. The Section put considerable effort into refereeing the statistical aspects of papers published in Cancer Research. Dr. Pettigrew, Dr. Connor, Dr. Gart and Mr. Nam refereed for the Journal of the National Cancer Institute. Dr. Gart refereed for Toxicology and Applied Pharmacology.

Publications

Kagan, A.R., Hafermann, M., Hamilton, M., Pierce, R., Morton, D. and Johnson, R.: Etiology, diagnosis, and management of pericardial effusion after irradiation. Radiologia Clinica et Biologica, (in press).

Kagan, A.R., Hamilton, M.A. and Bryant, T.L.: Radiosensitivity of *in vivo* mouse bone marrow cells. Radiologia Clinica et Biologica (in press).

Peterson, D.R., Thompson, D.J. and Nam, J.: Water hardness arteriosclerotic heart disease and sudden death. *Amer. J. Epid.*, 92: 90-93, 1970.

Slivka, J., Cargille, C.M. and Ross, G.T.: An analysis for superimposed biological rhythms: application to FSH and LH in women. *Am. J. Physiology* (in press).

Whang-Peng, J., Henderson, E.S., Knutsen, T., Freireich, E.J. and Gart, J.J.: Cytogenetic studies in acute myelocytic leukemia with special emphasis on the occurrence of Ph^1 chromosome. *Blood*, 36: 448-457, 1970.

Serial No. NCI-4267
1. Biometry Branch, OA&DD, Etiology
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Research in Mathematical Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Robert J. Connor, Hugh M. Pettigrew

Other Investigators: Jun-mo Nam, Donald G. Thomas, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	4.0
Professional:	3
Other:	1

Project Description

Objectives:

To conduct basic research in mathematical statistics, probability and applied mathematics; to develop new statistical methodology which is especially appropriate to biomedical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and test approximations by simulation techniques.

Major Findings:

The research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability and applied mathematics. These are summarized below.

1. The Comparison of Proportions.

John J. Gart has completed a long review paper on this subject which was an invited paper at an international conference and will soon be published in the *Review of the International Statistical Institute*. A second paper on approximate confidence limits for the odds ratio in combining several 2 x 2 tables was published in *Biometrika*. Computer programs implementing the latter method were written by Alroy M. Smith.

2. Large Population Epidemic Models.

Hugh M. Pettigrew has continued his research into deterministic and stochastic epidemic models, deriving new results which significantly extend the area of application of epidemic theory. A portion of this work concerning the so-called simple stochastic epidemic model and extensions of the Pettigrew-Weiss large-population epidemic model was submitted to the George Washington University in partial fulfillment of the degree of Doctor of Philosophy in the field of mathematical statistics. Dr. Pettigrew is now in the process of preparing this work for publication.

3. Grouping and Testing for Trend with Binary Data.

Robert J. Connor is investigating the effect of grouping in testing for possible linear relationship between a binomial variate and a covariate such as age.

4. Exact Confidence Limits for Common Odds Ratio.

Donald G. Thomas is currently developing a computer program to compute exact confidence limits for the common odds ratio in combined 2 x 2 tables.

5. Optimum Sample Size for the Control and Treatment with Consideration of Cost.

Jun-mo Nam has investigated the optimum sample sizes of the control and treatment groups for testing the difference between two survival times or proportions with consideration of cost. The comparison between the optimum and equal sizes design is made, and it indicates the former is greatly preferred when the difference between the cost for control and that for treatment per animal is large. The ways to compute the power of the test for the given total cost, or the optimum sample sizes and total cost for the specified power of test are illustrated. A paper on the subject has been submitted to Biometrics.

6. Grouping in Estimating Logistic Response Curve.

Martin A. Hamilton investigated the large and small sample properties of minimum logit chi-square estimators and maximum likelihood estimators where grouping of doses is employed to overcome the difficulties of analysis when only a single zero or one observation is available at each dose point in the ratio data. A paper on the subject has been submitted for publication.

7. Matching in Comparing Proportions and the Optimum Choice of Dosages in an Assay Design.

Jun-mo Nam has completed a submitted paper on the first of these projects and the second is just underway.

8. Approximate Confidence Limits for the Relative Risk.

John J. Gart and Donald G. Thomas completed a comprehensive review paper on a comparison of several proposed approximate methods for finding confidence limits for the odds ratio or relative risk. An adaptation of existing methods was proposed and shown to be much superior in many cases to previous methods commonly used. This paper should be of considerable benefit to researchers who do not have access to the sophisticated computing equipment required to obtain the exact solution. This paper has been submitted to the Journal of the Royal Statistical Society for publication.

9. Interaction in Contingency Tables.

Donald G. Thomas completed programs for 2 x J x K contingency tables which compute tests of the hypotheses of zero two-factor interaction and no difference in main effects.

10. Exact Statistical Tests in Bioassay.

John J. Gart has been investigating exact tests, exact power and confidence intervals for comparing quantal dosage response curves. Usually approximate methods are used; these exact methods can be used to validate the approximate tests particularly when only small numbers are available.

11. Chain-Binomial Epidemic Models.

John J. Gart has extended the Reed-Frost epidemic models to the case where several kinds of susceptibles are distinguishable. This permits statistical estimation and testing by maximum likelihood methods. Dr. Gart will present an invited paper on this subject at the annual meeting of the American Statistical Association this Summer.

Significance to the Program of the Institute

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI or other workers on problems in cancer research. Others are mathematical models which may elucidate the biological phenomena involved in cancer research and which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects described in the Major Findings will be continuing. Such subjects as exact statistical methods in bioassay and epidemic theory will be investigated by various members of the section. Other problems, not referred to above, shall also be attacked in the upcoming year; they will include the development of new statistical methods and mathematical models in various biomedical problems.

Honors and Awards

John J. Gart presented an invited paper entitled "Mathematical models in the interpretation of interaction between infective agents" at the WHO sponsored Symposium on Quantitative Epidemiology in Moscow, U.S.S.R. in November 1970. Presented an invited paper entitled "The comparison of proportions: a review of significance tests, confidence intervals, and adjustments for stratification" at the Seventh International Biometric Conference in Hannover, Germany in August 1970. Named Visiting Scholar at the Virginia Polytechnic Institute and State University for 1971. He presented lectures there in May 1971. He gave an invited lecture in the Department of Statistics of the Pennsylvania State University in October 1970. He continued to serve as a member of the Regional Advisory Board of the Biometric Society. Served as a member of an ad hoc Review Committee for Biostatistics Fellowships.

H. M. Pettigrew was awarded his Ph.D. in Mathematical Statistics from George Washington University in February 1971. Dr. Pettigrew served as a consultant to the Computer and Biomathematical Study Section of the Division of Research Grants. Dr. Pettigrew and Dr. Gart served as tellers for the election of officers for the Eastern Region of the Biometric Society.

Several members of the section served as referees for various statistical journals. Dr. Pettigrew and Dr. Gart refereed for Biometrics. Dr. Hamilton and Dr. Gart refereed for The Journal of the American Statistical Association. Dr. Connor and Dr. Gart served as reviewers for Science Books: A Quarterly Review. Dr. Gart also refereed for Technometrics.

Publications

Gart, J.J.: On the use of mathematical models in the interpretation of biomedical data. In Wasan, M.T. (ed): Proc. Symp. on the Mathematical Aspects of Life Sciences,pp.126-145,Kingston, Ontario, Queen's University Press, 1971.

Gart, J.J.: Some simple graphically oriented statistical methods for discrete data. in Patil, G.P. (ed): Random Counts in Scientific Work, Vol. 1, Random Counts in Models and Structures, 171-191, University Park, Penn., Penn. State Univ. Press, 1970.

Gart, J.J.: Point and interval estimation of the common odds ratio in the combination of 2 x 2 tables with fixed marginals. Biometrika 57: 471-475, 1970.

Gart, J.J.: On the ordering of contingency tables for significance tables. Technometrics 13:(in press).

Gart, J. J.: The comparison of proportions: a review of significance tests, confidence intervals, and adjustments for stratification. Rev. Int. Statis. Inst. 39: (in press).

Gart, J.J.: Mathematical models in the interpretation of the interaction between infective agents. Adv. Appl. Prob. 3: (in press).

Gart, J.J. and Pettigrew, H.M.: On the conditional moments of the k-statistics for the Poisson distribution. Biometrika 57: 661-664, 1970.

Pettigrew, H.M.: On the Mathematical Theory of Epidemics with Two or More Types of Infectives. Ph.D. dissertation, The George Washington University.

Slivka, J.: Some density functions of a counting variable in the simple random walk. Skandinavisk Aktuarietidskrift, 53: 51-57, 1970.

Slivka, J.: A one-sided nonparametric multiple comparison control percentil test: treatments versus control. Biometrika 57: 431-438, 1970.

Slivka, J. and Severo, N.C.: On the strong law of large numbers. Proc. Amer. Math. Soc., 24: 729-734, 1970.

Thomas, D.G.: Exact confidence limits for the odds ratio in a 2 x 2 table. Applied Statistics, 20: (in press).

Thomas, D.G.: Tests of fit for a one-hit vs. two-hit curve. Applied Statistics, 20: (in press).

Thomas, D.G. and Gart, J.J.: The small sample performance of some estimators of the truncated binomial distribution. J. Amer. Statist. Assoc., 66: (in press).

Serial No. NCI-4268
1. Biometry Branch, OASDD, Etiology
2. Special Cancer Survey Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Third National Cancer Survey

Previous Serial Number: Same

Principal Investigators: Harvey Geller and S.J. Cutler

Other Investigators: Susan S. Devesa, Kenneth Hurst, Constance Percy, Joseph Scotto, Theodore Weiss, John L. Young

Cooperating Units: University of Alabama School of Medicine
Georgia Regional Medical Program
State of Colorado Department of Health
University of Texas Southwestern Medical School
Michigan Cancer Foundation
University of Iowa
University of Minnesota, Department of Epidemiology, School of Public Health
University of Pittsburgh, School of Public Health
Puerto Rico Commonwealth Health Department
State of California, Department of Public Health
Iowa State University
Epidemiologic Pathology Unit, NCI
Electronic Data Processing Staff, BB, NCI

Man Years:	
Total:	11.5
Professional:	6.5
Other:	5.0

Project Description

Objectives:

To prepare a complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-1971, plus all other cancer cases seen by doctors or hospitals in those areas; to obtain demographic data and a description of the tumor for each patient, and to study certain economic, social, and other aspects of the disease in a sample of patients.

Extensive use is being made of computer capabilities in the processing of the survey documents. Each document is processed separately with the computer tagging and combining all the records for an individual patient. The computer is conducting a large number of edit checks -- consistency among documents on the same patient, as well as among items on the same document. The sample selection is also a computer operation with the printing of the appropriate labels included in the operation. The data processing time has been reduced to a minimum. Within five weeks after a record is received at NCI, the data are converted onto computer tapes, edit runs are completed and printouts of error reports and updates of survey control reports are mailed back to the field offices.

Major Findings:

None yet available.

Signficance of the Program to the Institute:

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence rates. Such data have countless uses once they are collected; of particular interest to NCI will be detailed studies of the relation between cancer incidence and possible etiologic factors. These include occupation, residence history, place of birth, and many other items included in the survey. Other products of the survey that will be important to the mission and program of NCI will be studies on the delivery of medical care to a large and representative sample of cancer patients, delays in diagnosis and treatment, the direct and indirect costs of cancer (especially as related to type of cancer, stage at diagnosis, family income, and geographic area), and the extent and duration of disability from cancer. The data can also be used in the setting of priorities within the Institute. The magnitude of the problem coupled with the economic factors provide material for cost effective determinations which can be taken into consideration in planning areas of effort.

The survey will also produce unique data which can be used by other investigators as a starting point for more intensive investigations. One area of special interest is rare tumors. Because of the low numbers of these tumors, epidemiologists feel that investigations will yield significant results and possible etiological leads. The survey will be the only mechanism available for identifying large enough populations of these rare tumors for epidemiologic investigation, at a time when additional data can be obtained.

Proposed Course of the Project:

Because of the delays in hiring of the necessary personnel both at NCI and in the Field Offices, all of the areas did not start on survey operations at the start of the year. Effort will have to be directed to get up to date as soon as possible and develop a pattern of operation that is most efficient. Limitations on staff also led to delays in finalizing the forms that are to be used with the sample population. All of the interview schedules and the training programs for the interviewers will be completed by the end of FY70. Once this is completed the staff of the Special Cancer Survey Section will be almost entirely concerned with supervising the operation of the program in all its aspects. Channels and means of communication between the administrative office and the 11 field offices are being developed to insure consistency and comparability of the data being collected. Periodic workshops including personnel of the section and the field offices will be held at regular intervals. Coordination of the 11 field offices with the administrative office in this section and with the data processing operation elsewhere in Bethesda continues to be a major undertaking, and will involve very extensive travel on our part.

Despite the delays in the start of the data accumulation operation, much progress has been made. As of the end of 1971, over 350,000 records have been processed and are on computer tape. There is some backlog in a number of field offices that has to be abstracted which should be accomplished by the end of the fiscal year. Therefore, it is anticipated that at the start of 1972, all the abstracting operations will be current. The sample portion of the survey was delayed more than six months due to limitations of NCI staff available in the finalization of the forms. The interview portion of this program is picking up momentum and it also should be on a current basis by the start of FY 1972.

The implementation of the computer system was developed over a full year. Although this did not directly affect the abstracting procedure, it had a direct affect on the method of operations. All of the random picking of samples, and the preprinting of some of the data, had to be done by hand. The computer editing was also delayed so that there was a very large number of corrections that had to be made at the field office in a very short space of time, rather than be spread over most of the year.

The delay and debugging of the computer system also meant that listings to assist the field offices were not available. This made the work more difficult and time consuming. The system is almost fully operational at the present time, and only the minor bugs remain to be eliminated. This will greatly facilitate the field office operations and production should improve.

Personnel problems at some of the field offices also had an adverse affect on the production of records. The inability to hire qualified personnel under the Philadelphia civil service regulations necessitated the dropping of this area from the survey. In several field offices there have been changes in the supervisor. The personnel situation at every field office is under constant supervision by NCI staff and corrective actions are taken as soon as problems are identified.

Intensive cancer morbidity and mortality studies over a three-year period in this large population will lead to the first comprehensive picture of cancer in the United States in over 20 years. The economic and social investigations have never been attempted before in this way or on this scale. Complete coverage of population groups included in the new survey will provide physicians and public health officials with answers to important questions, such as variations in the incidence and prevalence of specific forms of the disease and the economic cost of cancer to a community. Physicians and hospital administrators in each area will also be able to plan treatment and care facilities more effectively.

Methods Employed:

In each study area the National Cancer Institute has contracted with local organizations to hire field staff and gather data. Data are being requested from doctors and obtained from hospital records, death certificates, and upon approval of their doctors, a sample of cancer patients are being interviewed. Local staffs do the preliminary editing and coding of data, and are responsible for disposition of the original records at the close of the survey. Institute staff in Bethesda, Maryland complete the editing and coding of data and convert it to magnetic tape for use in preparing detailed reports through electronic computers.

Basic information is being collected on every new cancer case, except certain skin cancers, during the 3-year period. This includes race, age, place and date of birth, sex, and marital status. Medical information includes the primary site and histologic type of tumor, date and method of diagnosis. Information available from any existing cancer register or continuing survey in a study area is being utilized to avoid duplication. All reports are treated as strictly confidential.

Death certificates mentioning cancer are obtained from the appropriate vital statistics office. Local survey staff or hospital personnel abstract information on cancer patients from hospital records.

Trained interviewers, after receiving written permission from attending doctors, obtain from a selected sample of patients information on the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions are also asked regarding religion, number of children, socioeconomic status, and personal habits such as smoking. Hospital records on this selected population are reviewed for more detailed information on cost of hospitalization, methods of meeting this cost, treatment for the cancer, and the extent of the disease at time of diagnosis.

The main product of this survey will be a detailed analysis of cancer incidence rates. There will also be several ad hoc reports on special problems, such as the relation between occupation and cancer incidence. Summary reports on each of the study areas will be prepared for the use of local physicians, hospitals, public health workers, and other interested persons.

Two workshops have been conducted during this fiscal year. These have been well received by the field office staff and have been a valuable means of instruction and communication among the field officers and between the field office and the Section staff.

Methods and procedures have been developed for Section editing of the abstracts, and the review of the medical portions of the abstracts. Periodic visits to the field office by Section staff have proved a valuable means of continued evaluation of the field office procedures and has resulted in an increase in the efficiency and completeness of the operations.

All quality control procedures have not been implemented. The reinterview by the State University of Iowa staff has been started. The reabstract program has been developed, and will be operational in the very near future. This will increase the travel of the Section staff since they are intimately involved with the reabstract program.

A good deal of staff time will be spent in the analyses and evaluation of computer tabulations, especially those based on the complete reporting for calendar year 1969. These will be useful mainly for operational purposes with no intent of any general distribution. These reports will also be useful for the stimulation of special studies since the data should indicate areas of potential.

Honors and Awards

None

Publications

None

Serial No. NCI-4269
1. Biometry Branch, OASDD, Etiology
2. Automatic Data Processing Management Section (ADPMS)
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Data and Information Processing Consultation and Support

Previous Serial Number: None

Principal Investigator: Theodore Weiss

Other Investigators: T. D. C. Kuch, James E. Larson, Anne Baranovsky, Norman Oslik

Cooperating Units: None

Man Years:
Total: 6.5
Professional: 4.5
Other: 2.0

Project Description

The Automatic Data Processing Management Section provides consultation and support to the scientific and administrative staff of the Etiology Area of the Institute. It has expertise in applying data and information processing technologies to epidemiologic, laboratory, and clinical research projects, program planning and appraisal activities, and administrative management operations.

Objective:

To facilitate the research effort by minimizing the mechanical and logistical constraints upon investigators arising from their activities involving data and information collection, conversion, purification, computation, storage, retrieval, analysis, and dissemination.

Methods Employed:

Consultation and support is provided through three disciplinary areas:

> A Planning and Development Unit responsible for: (1) participating in the planning aspects of research projects which have data and information processing problems; (2) conducting studies and disseminating

information on the feasibility of using computer-related techniques, such as information storage and retrieval, computer-related proprietary software packages, such as the UCLA Biomedical Data Processing (Statistical/Mathematical) Sub Routine Package (BMD), and computer-related equipment, on ongoing and future projects and operations; and (3) developing standards and monitoring performance relating to the quality of data and information processing products and services provided by the ADPMS, commercial contractors, and research contractors;

A Systems Design and Implementation Unit responsible for providing technical support in the form of detailed systems analysis, systems design, computer programming, implementation, and maintenance of data and information processing systems, which use digital computers, scientific computers with analog-digital converters, and related electronic instrumentation; and

An Operations Unit responsible for converting raw data into forms appropriate for computer processing, the day-to-day coordination, liaison, and operation of data processing systems, and the operation of such ADP equipment as the ADPMS has.

Major Findings:

The Automatic Data Processing Management Section was formally established in February 1971, having existed previously as a staff function in the Office of the Chief, Biometry Branch.

During the past year most of the Section's resources were dedicated to four continuing, large-scale Demography Area studies: the Third National Cancer Survey; the Japanese Migrant Studies; the Veterinary Medical Data Program; and the Study of Retired Railroad Employees. The Section also began supporting two new projects: a collaborative epidemiologic and clinical study of cancer risks in Japan and Hawaii, which involves the design and implementation of an automated data collection and processing system for data derived from patient interviews, clinical examinations, and laboratory tests; and participation in the development of a Program Monitoring System for the Associate Scientific Director for Program to facilitate Etiology Area-wide program planning and related contract activities. In addition, some work has been done to automate activities in the Administrative Office.

In May 1971, the ADPMS acquired a task-order type contract with a commercial data processing contractor who has particular expertise in supporting biomedical research. This has been done in order to provide the Etiology Area with data processing systems, for which the demand fluctuates according to the research requirements. Particular emphasis has been given to selecting a data processing contractor capable of providing support to the Viral Oncology and Carcinogenesis Areas. Subsequently, members of the ADPMS began preliminary discussions with investigators in these areas who have requested assistance. It is anticipated that the Section will begin a growing level of support here in the next fiscal year.

Significance to the Program of the Institute:

The systematic capture, organization, and display of complex and diverse data is of considerable importance in the planning, conduct, and management of the research efforts. As multidisciplinary and collaborative activities increase in scope and complexity, the problems of data linkage, ability to manipulate, analyze, and communicate large quantities of data and information could become unmanageable without the assistance of computer-related technologies.

Proposed Course of the Project:

As a relatively new resource in the Etiology Area, the ADPMS should gradually develop the expertise and associations required for it to be responsive over a broad area of biomedical and biostatistical research.

Honors and Awards

Kuch, T. D. C.: Reviewer for "Computer Analysis of Labor Progression" paper for publication at request of Dr. Emanuel A. Friedman, Department of Obstetrics and Gynecology, Harvard Medical School, and Mr. Bernard Kroll, Section on Systems Design and Data Processing, Office of Biometry, National Institute of Neurological Diseases and Stroke.

Weiss, T.: Member of a Contract Review Committee, American College of Surgeons, Chicago, Illinois. Member, Grant Application Review Committee, Special Center of Research, UCLA Medical School, Harbor General Hospital, Torrance, California. Member, Grant Application Review Committee, Special Center of Research, School of Medicine, University of Washington, Seattle, Washington. Member, Contract Review Committee, at the request of Dr. Robert I. Levy, Chief, Lipid Metabolism Branch, NHLI.

Publications

None

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

Office of the Chief

HEALTH INSURANCE PLAN OF GREATER NEW YORK (69-88)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Mr. Sam Shapiro

Project Officer (NCI): Mr. William Haenszel

Objectives: A. To determine whether a simple screening technique (x-ray mammography) can diagnose breast cancer early, and

B. To determine if the very early diagnosis of breast cancer is really useful in improving long term survival.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving mammography at annual intervals and a control group of 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled, "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966.

During one reexamination cycle 40 cc of blood was taken from women in the study population and stored.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes mammography. Of 125 breast cancers diagnosed to date among women in the study population, 43 would have been missed in the screening examination if mammography had not been used. The proportion of breast cancers detected on screening with no evidence of nodal involvement is high -- more than 70 percent, compared with 42 percent for women in the control population. Preliminary data on mortality point to the prognostic advantage of early diagnosis. A total of 21 deaths have occurred to date in the study population, 18 due to breast cancer, compared with 34 deaths in the control population, 32 due to breast cancer.

Significance to NCI Program & Bio-Medical Research: Early diagnostic procedures may be of use in the early treatment and potential cure of cancer patients. If it develops that mammography is such a technique, it then could be extended to other populations and would then have the effect of reducing the mortality from breast cancer within the United States.

Proposed Course of Project: The screening part of the study is complete and the emphasis from now on will be on follow-up. These women will be followed for as long a time period as necessary to demonstrate whether the technique has any usefulness as an early diagnostic device or whether its usefulness is limited. The duration of the study is contingent upon the answers to the questions implied by the objectives.

Supplemental lines of inquiry may be suggested by the Breast Cancer Task Force. Proposals for use of the stored blood plasma are being developed.

Date Current Contract Initiated: 11-1-62

Current Annual Level: $203,100

NORWEGIAN PUBLIC HEALTH SERVICE (64-499)
UNIVERSITY OF MINNESOTA (66-919)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (64-499)
Dr. Leonard Schuman (66-919)

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine the reason for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic-demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: The findings to date come from the cardiorespiratory symptom data and two years of mortality follow-up in the United States on the cohorts of British and Norwegian-born.

A striking feature of the symptom data was the low prevalence of respiratory symptoms among British migrants in the United States whose experience was comparable to that for the U.S. native-whites and far below the prevalence rates reported in the United Kingdom. The symptom prevalence for British migrants varied only slightly with respect to age at time of migration and place of birth and residence in the United Kingdom. The symptom data were consistent with the evidence provided by mortality data. The symptom data were also highly correlated with subsequent mortality experience in the study cohorts. It was also found that migrants coming to the U.S. prior to age 15 had the higher risks for coronary heart disease. The limited numbers of deaths for individual cancer sites do not permit firm statements at the present time on effects associated with age at migration.

NORWEGIAN PUBLIC HEALTH SERVICE (64-499)

In Britain, Norway and the United States respiratory symptoms are strongly associated with smoking history. In heavily polluted English cities urban exposure and smoking history interact to produce respiratory symptom prevalence greater than would be expected from the sum of the two separate effects. Similar interactions are not found in Norwegian cities, where air pollution levels are lower.

Significance to NCI Program & Bio-Medical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: The Norwegian Public Health Service is responsible for conduct of all phases of data collection in Norway carried out in connection with the Norwegian-British Migrant Study concerned with cancer and cardiorespiratory diseases. Symptom and demographic data have been collected for a representative sample of the Norwegian population and from brothers and sisters of migrants to the United States included in the U.S. study samples. The Norwegian cohorts will be followed for cancer incidence and mortality data. A companion program of case-control interviews for gastrointestinal cancer is underway in Norway and will be extended to other sites at a later date.

The University of Minnesota has defined a cohort of approximately 20,000 policyholders in an insurance company that has a high proportion of persons of Norwegian descent and will monitor the group prospectively for mortality data. The contractor has also carried out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with ongoing work in Norway. One investigator from the Cancer Registry of Norway is in residence almost continuously at the University of Minnesota to assure close liaison.

Date Contract Initiated:	March	1964 (64-499)
	June	1966 (66-919)
Current Annual Level:	$47,010	
	$20,000	

TOHOKU UNIVERSITY SCHOOL OF MEDICINE (64-10)
UNIVERSITY OF HAWAII (63-558)
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (64-873)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Professor Mitsuo Segi (64-10)
Dr. Richard K. C. Lee (63-558)
Dr. John E. Dunn, Jr. (64-873)

Project Officer (NCI): Mr. William Haenszel

Objectives: The purpose of this contract is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The purpose is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques are employed, basically using a system of diagnosed cases and matched controls. In addition data have been collected from samples of Japanese households. The matched case-control techniques is particularly useful in this kind of study. Pathology protocols are being completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis is being placed on the correlation of the epidemiological and pathology findings. In preparation for later case-control studies of prostate cancer, latent prostate cancer is being studied in 5 groups of patients.

Major Findings: The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach, while little change has been observed for breast. To date, the potential information exists tending to incriminate changes in dietary habits and in smoking habits for the changes in stomach cancer, and in lung cancer in the Japanese populations. Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. Japanese migrants to Hawaii have an intermediate position as to prevalence but show a complete shift in histology from "Japanese" to "American" type.

Significance to NCI Program & Bio—Medical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicates those that are predominantlyenvironmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project: Interviews of household samples in Hiroshima and Miyagi prefectures, California and Hawaii have been completed. Interviews of stomach cancer patients and matched hospital controls in Japan have been completed but will continue to be collected in Hawaii and California. The

TOHUKU UNIVERSITY SCHOOL OF MEDICINE (64-10)
UNIVERSITY OF HAWAII (63-558)
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (64-873) (continued)

difference in findings for Issei and Nisei require the continued collection of histories in the United States. Current work covers case-control interviews for cancer of the esophagus, rectum, lung and leukemia. Other sites will be added, including breast, cervix and prostate as work progresses. Acquisition of pathology protocols on diagnosed cases has been coordinated with the collection of epidemiologic data. Arrangements have been made to collect pathology protocols for colon, rectum, esophagus, gall bladder and liver. In Hawaii, case-control data will be complemented by parallel prospective data collected by the NHLI in their Honolulu Heart Program Study of a cohort of Japanese men. This program will be expanded to cover cancer and precancer by supplementing NHLI support. Pathology studies on this group of case-control material will include more intensive study of surgical and autopsy tissue and bacteriological and biochemical characterization of patients and controls.

Date Contract Initiated:	July	1964 (64-10)
	April	1963 (63-558)
	May	1964 (64-873)

Current Annual Level:	$60,000 (64-10)
	$44,670 (63-558)*
	$32,838 (64-873)

* Pathology support to this contract provided by Kuakini Hospital under contract 65-1003 in the amount of $17,473.

UNIVERSIDAD DEL VALLE -- FACULTAD DE MEDICINA (PH43-66-907)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Professor Pelayo Correa

Project Officers (NCI): Mr. William Haenszel, Dr. John Berg

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia and to correlate the morbidity data with pathology findings.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socioeconomic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys on a limited scale are being undertaken in Cartegena and Medellin, cities thought to have greatly different site-specific incidence.

Studies of autopsy materials and surgical specimens are being conducted in Cali to define criteria which differentiate high-risk and low-risk populations.

UNIVERSIDAD DEL VALLE -- FACULTAD DE MEDICINA (PH43-66-907)

In Cali an organ bank of tissues from persons dying from accidents and acute diseases is being utilized for study of occult tumors and malignant precursors with emphasis on stomach, colon and cervix. The organ bank findings also emphasize the distribution by place of birth and are used to complement the incidence data from the morbidity surveys. Similar pathologic material is being collected in New Orleans and Hawaii.

Major Findings: Preliminary observations on medical examiner and hospital autopsy materials provide some provocative leads. Application of the Lauren concept of intestinal and diffuse types of stomach cancer points to substantial differences in distribution between Cali where stomach cancer is common and in Cartegena where it is rare. Intestinalization has also been found to be much more frequent in stomachs from persons migrating from areas with high stomach cancer risks (Narino). The vital statistics for Cali have consistently described a low mortality for prostatic carcinoma in the presence of a high incidence rate. Review of the pathology materials indicates the majority of these cancers were found during transurethral resection for apparent hypertrophy and showed diffuse cancer or an early malignancy in the prostate ducts, which is quite contrary to American experience and raises a question of a different type of disease.

Significance to NCI Program & Bio-Medical Research: The Department of Pathology at the Universidad del Valle is strongly oriented to epidemiology and geographic pathology and its activities have no counterparts in U.S. university departments of pathology. The objective in Colombia is to produce data for comparative studies in the United States and elsewhere, with emphasis on those areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: The long-range objective is the correlation of morbidity and pathology findings and the search for etiologies. In the immediate future, collection of data on gradient of cancer risks within Colombia by socioeconomic class, place of birth and current residence, etc. will be continued. Much effort will need to be placed on refinement of pathology criteria and their test as discriminants between high- and low-risk areas. Priority in study of the pathology materials will be given to stomach, colon, cervix, and thyroid.

Date Contract Initiated: June 1966

Current Annual Level: $46,764

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

END RESULTS SECTION

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (69-5)
UNIVERSITY OF CALIFORNIA (67-62)
CHARITY HOSPITAL OF LOUISIANA (64-83)
UNIVERSITY OF CHICAGO (65-5)
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)
INDIANA UNIVERSITY HOSPITAL(70-2000)
STATE UNIVERSITY OF IOWA (64-15)
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (64-13)
UNIVERSITY OF MICHIGAN (66-24)
UNIVERSITY OF VIRGINIA (67-13)

Title: End Results Evaluation

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: To obtain and analyze volume data for all cancer diagnoses, with analysis of specific types of cancer in patients with selected characteristics, in order to study:

1. the changes over time in the use of different methods of therapy

2. the relative effectiveness of the various treatment modalities as judged by patient survival, and any changes over time in survival.

To acquire and analyze supplementary data designed to explain further any specific finding from the relatively crude mass data.

Methods Employed: The National Cancer Institute sponsors a program in which more than 100 hospitals contribute data on the end-results of cancer treatment. The Biometry Branch has professional and technical responsibility for supervising these contracts. Information on individual patients is submitted to the Biometry Branch, according to a prescribed schedule, and in accordance with a uniform punchcard code. This program makes available to the National Cancer Institute information on a very large series of patients (e.g., 460,000 patients were first treated in participating hospitals during the period 1940-1964) in a form which permits maximum flexibility in handling and analyzing the data, and also provides an extensive resource for special studies on selected series of cases. Partial support for three centralized registries (California, Connecticut, and Massachusetts) and for nine registries in university hospitals is provided through the contract mechanism to make it possible for the participating registries to meet specified criteria regarding completeness, currency, and accuracy of information.

To obtain supplementary data, patients with pertinent characteristics are identified in the accumulated files. The medical records for these patients are reabstracted in detail using specially designed protocols. The records of similar patients may be abstracted on a prospective basis.

Major Findings: A firm answer to the question: "How many patients are being cured?" is unavailable because cancer can recur many years after treatment. However, a long-term survival rate provides a meaningful measure of therapeutic success. The 20-year rate adjusted for normal mortality expectation, is such a measure. Available data on patients treated since 1940 permit projection of 20-year adjusted survival rates for patients treated during the 1960's. The estimated proportion of successfully treated patients (20-year adjusted survival rate) is encouragingly high for those with resectable tumors and no evidence of spread beyond the site of origin, e.g., 85% for uterus, 75% for ovary, 60% for colon, rectum, breast and bladder.

Significance to NCI Program and Bio-Medical Research: The End Results Program provides the most comprehensive information available on major trends in cancer treatment and associated results, and provides a base for a variety of special studies.

Proposed Course of the Project: Increased attention will be given to reducing the time lag in reporting incidence data and in reporting survival data for disease entities for which therapeutic practice is changing rapidly, e.g., leukemia and Hodgkin's Disease. Greater emphasis will be placed on developing additional population-based registries to provide a resource for a wide variety of epidemiologic and clinical studies.

	Date Initiated	Current* Annual Level
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (69-5)	7-1-62	$265,000
UNIVERSITY OF CALIFORNIA (67-62)	11-1-66	16,373
CHARITY HOSPITAL OF LOUISIANA (64-83)	10-1-63	31,834
UNIVERSITY OF CHICAGO (65-5)	7-1-64	28,642
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)	6-1-63	112,542
INDIANA UNIVERSITY HOSPITAL (70-2000)	7-1-63	44,217
STATE UNIVERSITY OF IOWA (64-15)	7-1-63	27,365
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (64-13)	7-1-63	60,000
UNIVERSITY OF MICHIGAN (66-24)	9-1-65	30,870
UNIVERSITY OF VIRGINIA (67-13)	7-1-66	28,164
YALE UNIVERSITY DEPARTMENT OF PUBLIC HEALTH (70-2287)	6-29-70	52,961

*(1970-71 estimate)

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

Special Cancer Survey Section

IOWA STATE UNIVERSITY (Ames) (69-94)

Title: Reinterview Program, Third National Cancer Survey

Contractor's Project Director: Dr. T. A. Bancroft

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: To provide continuous monitoring of the quality of data collected in interviews of a sample of patients in the Third National Cancer Survey. This will be primarily by a re-interview of a sub-sample of interviewed patients.

Major Findings: No data yet collected

Significance to Bio-Medical Research and the Program of the Institute: See Project Report NCI-4268.

Proposed Course: Reinterviews have been stopped. A comparison of the reinterviews with the original interviews is under way, but due to the small number of cases involved little can be expected in the way of analytic results.

Date Contract Initiated: 2-15-69

Current Contract Level: 2-15-69 - 6-30-71: $28,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

Special Cancer Survey Section

MAUCHLY AND COMPANY, INC. (70-2062)

Title: Maintenance of Third National Cancer Survey Data Processing System

Contractor's Project Director: Thomas Marshall

Project Officer (NCI): Theodore Weiss

Objectives: The data processing system for the Third National Cancer Survey has been developed. This contract provides for the systems design and computer programming services necessary to implement and maintain the system.

Major Findings: The Third National Cancer Survey data processing system is a large and complex system because of the characteristics, objectives and requirements of the Third National Cancer Survey itself. Five separate functional groups have processing responsibilities in the Survey. The entire system was designed to minimize the extent of clerical and professional manpower required in the Survey. The computer portion of the system was designed to process approximately one and one-half million documents in twelve different variable-length formats over a four-year period. One document is a multi-page form itself. Extensive document control, data editing and cross-checking of some five hundred data items are performed routinely. The system assigns random numbers to all new cases, selects two samples of the cases, and partially preprints forms for more extensive data collection, selects a sample of the cases for quality control reabstracting, identifies probably mismatched patient records, identifies cases with missing documents as well as cases with documents missing data items. It periodically notifies the field offices when their submission of certain documents is delinquent, and when errors in the files have not been corrected within a reasonable period of time. It produces a variety of productivity reports. It also provides a simple and accurate method of correcting errors on the very large and complex files, and provides many different computer printouts to assist the data collection centers in their activities. It makes all machine-processed data available for analysis during the Survey, and allows all data collected in the Survey to be available for analysis within a relatively short time after completion of machine processing.

Significance to Bio-Medical Research and the Program of the Institute:
See Project Report NCI.

Proposed Course: It is proposed to keep the contract going until the system is fully operational, including the addition of special data collection forms, and the production of routine tabulations for preliminary analysis of incoming data.

Date Contract Initiated: 12-22-69

Current Contract Level: 12-22-69--6-21-71
$184,155

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1970

Special Cancer Survey Section

UNIVERSITY OF ALABAMA MEDICAL CENTER (Birmingham) (69-49)
CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (Berkeley) (69-51)
COLORADO DEPARTMENT OF PUBLIC HEALTH (Denver) (69-50)
COMMONWEALTH OF PUERTO RICO, DEPARTMENT OF PUBLIC HEALTH (San Juan) (69-72)
IOWA, UNIVERSITY OF (Iowa City) (69-42)
MEDICAL ASSOCIATION OF GEORGIA (Atlanta) (69-47)
MICHIGAN CANCER FOUNDATION (Detroit) (69-41)
UNIVERSITY OF MINNESOTA, SCHOOL OF PUBLIC HEALTH (Minneapolis) (69-45)
UNIVERSITY OF PITTSBURGH (69-43)
UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL (69-40)
MAYO FOUNDATION (Minnesota) (70-2057)
YALE UNIVERSITY, DEPARTMENT OF PUBLIC HEALTH (70-2287)

Title: Third National Cancer Survey

Contractor's Project Director: See table on following page

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: Procurement and initial processing of data for the Third National Cancer Survey.

Major Findings: Data have just begun to accumulate and no analysis has been attempted.

Significance to Bio-Medical Research and the Program of the Institute: See Individual Project Report NCI-4268.

Proposed Course: In spite of some severe delays in implementing various aspects of the program, the survey is now running rather smoothly. As of the end of February 1971, approximately 345,000 documents had been submitted to the NCI central office from the 11 field offices. The computer processing and edits are operational except for some of the sample study documents. Several modifications to working procedures have been made with increased efficiency of use of personnel time.

An intensive effort has been undertaken to complete case findings for the cases diagnosed during 1969. Field office personnel are checking all possible sources in each of the hospitals to ensure that each reportable case is identified and a document submitted. This is resulting in the discovery of some large holes in our previous operation with the identification of many missed cases. In addition, intensive efforts are being made to follow-back on cases for which only death certificate information is available in order to acquire information on date of diagnosis and site. The resultant workload has forced some of the field offices to curtail some of their other activities, such as parts of the sample studies, as personnel are diverted to the more pressing jobs of case finding and abstracting.

The delays in completion of the computer system has meant that, as sections of the system were implemented, the field office received large error listings or edit outputs working off large backlogs all at once, rather than on an ongoing schedule. Also, various listings to assist the field offices in their work have not been available to the extent desired. The system is almost fully operational at the present time, and only minor bugs remain to be eliminated. This will greatly facilitate the field office operations and production should improve.

Two workshops have been conducted during the fiscal year. These have been well received by the field office staff and have been a valuable means of instruction and communication among the field officers and between the field office and the Section staff.

Periodic visits to the field offices by Section staff are a key quality control element and have resulted in an increase in the efficiency and completeness of operations.

The reabstracting program is currently undergoing a temporary halt due to the concentration on case findings. Reabstracting of a sample of hospital records by central office staff has been shown to be an effective method of identifying problem areas in the interpretation of survey rules and will be reinstituted as soon as time permits. The sample reinterview program, on the other hand, has not been carried out effectively by the contractor.

Plans are being made for the issuance of preliminary data on 1969 cases starting October 1, 1971. As the analysis of this subset of survey data continues, problems in the data collection at present will be identified and corrected while it is still possible to do so. In addition, preliminary analysis will also provide the testing grounds for final analysis plans.

Increased attention will be given to selecting two or three survey areas for conversion to a continuing population based tumor registry. The goal is to develop several geographic areas as resources for a wide variety of epidemiologic and clinical research.

Date Contract Initiated: See table on following page

Current Contract Level: See table on following page

Contractor	Contractor's Project Director	Date Contract Initiated	Current Contract Period	Level
University of Alabama	Dr. Peter Peacock	10/1/68	9/1/70-8/31/71	$ 62,340
California Dept. of Public Health	Mr. George Linden	10/1/68	9/1/70-8/31/71	179,280
Colorado Dept. of Public Health	Dr. Mollahan	10/1/68	9/1/70-8/31/71	130,682
Puerto Rico Dept. Public Health	Dr. I. Martinez	12/9/68	12/9/70-12/8/71	9,049
University of Iowa	Dr. Mason	10/1/68	9/1/70-8/31/71	87,192
Medical Assoc. of Georgia	Dr. J. Cooney	10/1/68	9/1/70-8/31/71	82,500
Michigan Cancer Foundation	Dr. M. Brennan	10/1/68	9/1/70-8/31/71	122,160
University of Minnesota	Dr. L. Schuman	10/1/68	9/1/70-8/31/71	103,080
University of Pittsburgh	Dr. A. Ciocco	10/9/68	10/9/70-8/31/71	120,830
University of Texas	Dr. Eugene Frenkel	10/1/68	10/1/70-9/30/71	111,828
Mayo Foundation (Minnesota)	Dr. L. Kurland	12/22/69	12/22/70-12/21/71	25,000
Yale Univ. Dept. of Public Health	Dr. R. Greenberg	6/29/70	6/29/70-6/28/71	52,961

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

Special Cancer Survey Section

FARRINGTON SERVICE COMPANY (69-2247)

Title: Data Conversion for the Third National Cancer Survey

Contractor's Project Director: Frank Boudreau

Project Officer (NCI): Theodore Weiss

Objectives: Convert the data on approximately two hundred thousand Third National Cancer Survey documents to magnetic tape.

Major Findings: Data is being converted by the optical character recognition method (OCR). The contractor is using a system of procedures and computer runs specifically designed for him by the Institute. The system includes extensive security controls over documents and information, as well as a pre-edit of completed Survey documents, and manual and computer edit checks of the data transcription process.

Significance to Bio-Medical Research and the Program of the Institute: See Project Report NCI.

Proposed Course: Proposal is to be put out for bid to insure that continuation of the contract is at the lowest possible cost.

Date Contract Initiated: 6-30-69

Current Contract Level: 6-30-69--1-31-71
$173,692.00

CONTRACT NARRATIVE
BIOMETRY BRANCH, ETIOLOGY AREA
Fiscal Year 1971

Special Cancer Survey Section

MARYLAND NATIONAL OPTIMATION SERVICE, INC. (NIH-71-2050)

Title: Data Conversion for the Third National Cancer Survey

Contractor's Project Director: Carl Zelman

Project Officer (NCI): Theodore Weiss

Objectives: Convert the data on approximately five hundred thousand Third National Cancer Survey documents to magnetic tape.

Major Findings: Data is being converted by the optical character recognition method (OCR). The contractor is using a system of procedures and computer runs specifically designed for him by the Institute. The system includes extensive security controls over documents and information, as well as a pre-edit of completed Survey documents, and manual and computer edit checks of the data transcription process.

Significance to Bio-Medical Research and the Program of the Institute: See Project Report NCI.

Proposed Course: Proposal is to be put out for bid to insure that continuation of the contract is at the lowest possible cost.

Date Contract Initiated: 6-30-69

Current Contract Level: 10-19-70--1-31-73
$169,700

SUMMARY REPORT
EPIDEMIOLOGY BRANCH
JULY 1, 1970 through JUNE 30, 1971

The objective of the Epidemiology Branch is to test ideas concerning the origins of cancer through epidemiologic studies based on medical knowledge and experience and on an awareness of resources best available at the national or international level. An idea is developed from existing knowledge, which may come from the laboratory, the clinic, or other epidemiologic studies. Emphasis is placed on epidemiologic research based on important new information from laboratory investigations at the National Cancer Institute and elsewhere.

Staff Changes: Andrew G. Glass, M.D. resigned to accept a position as pediatrician with the Kaiser-Permanente system in Portland, Oregon. Miriam D. Manning, M.D. retired after 20 years in the Public Health Service. John J. Mulvihill, M.D. joined the Branch as Staff Associate. Wladimir Wertelecki, M.D. resigned to accept a position at the Medical College of South Carolina.

Outside Training: Frederick P. Li, M.D. became a Fellow in Oncology at the New England Center Hospital in Boston.

Research Program:

Childhood Cancer: In 1962 the Branch began to examine the relationship between cancer and congenital defects. This epidemiologic approach, little used previously, seeks an excessive coexistence of the two diseases in persons, families or communities. When diseases are found to occur together more often than can be expected by chance, each can be studied in the light of what is known of the other for clues to etiology. In the past year several interesting associations between cancer and congenital defects were identified.

A review of childhood teratomas revealed a relationship between sacrococcygeal teratoma and a variety of pelvic defects, including duplication of the pelvic organs in girls. A large series of children with retinoblastoma showed an increased frequency of mental retardation, often associated with a complex of malformations suggesting deletion of a D-group chromosome; this cytogenetic defect has been described in a few recent case reports of retinoblastoma. Children with retinoblastoma also had an apparent excess of double primary tumors, especially osteogenic sarcoma, which was not attributable to radiation. We reported a series of patients with Turner's syndrome, plus a review of the literature, which suggested an increased risk of neurogenic neoplasms (31). In a large multihospital survey of brain tumors, about 5% of the children had evidence of an underlying hamartomatous or neurocutaneous syndrome, but no other defects clearly exceeded expectation. A study of ovarian tumors reported to the Armed Forces Institute of Pathology suggested relationships between germ cell tumors and developmental defects of the neuraxis, and between gonadal-stromal tumors and subsequent thyroid disorders. A review of the relation of childhood cancer to congenital defects was published this year (17).

Among children with Down's syndrome (mongolism), leukemia was found to affect all cell lines to a similar extent (4,13). However, the age peak of leukemia in Down's syndrome occurred much earlier in childhood than usual; this shift probably accounts for the higher frequency of the myelogenous cell type (which prevails in the postnatal period) in case reports of leukemia with Down's syndrome. In a collaborative study with the Perinatal Research Branch, NINDS, a series of children with neonatal polycythemia showed a 19-fold increased frequency of Down's syndrome, suggesting the presence of an extensive myeloproliferative defect in newborns with this anomaly (30).

The Branch continued to conduct epidemiologic surveys of childhood cancers, based on multihospital studies of medical records and examination of death certificates for all children who died of cancer in the United States, 1960-67. Some surprising racial differences were noted. A marked deficiency of Negroes with Ewing's tumor, first identified in the mortality series, was confirmed by a review of cases diagnosed at the Armed Forces Institute of Pathology (11). In contrast, mortality from retinoblastoma was twice as high in Negro children than in whites, and carcinoma of the large intestine about 4 times greater in Negro children. Further studies are planned to determine whether the Negro excesses for these cancers reflect high incidence or case-fatality. Japanese children in the U.S., as in Japan, showed an increase in mortality from pineal tumors (21).

A previously reported 5-year mortality survey to detect sib aggregation of cancer was extended by 3 years (14). The number of presumably identical sets of twins who died of leukemia early in life increased from 5-7 pairs, maintaining the concordance rate at about 17%. Among sibs other than twins, brain tumor was found in 8 pairs vs. 0.9 expected. There were also 8 families vs. 0.9 expected in which one child died of brain tumor and another died of cancer of the bone or muscle. In two families, one child had adrenocortical carcinoma and another had rhabdomyosarcoma, a combination which represents part of a familial cancer syndrome. In another familial pattern, each of three children with lymphoma and one with lymphocytic leukemia had sibs with carcinoma of the colon. One of these families, from the NIH Clinical Center, was previously known to us; the affected children had polyposis of the colon, and two other first degree relatives died of sarcoma.

During the year many physicians brought our attention to children with neoplasms which seemed to be part of a familial multiple-cancer syndrome. We advised on and conducted many family studies. When possible, appropriate biological material was collected for distribution to various NCI laboratories, in an effort to identify mechanisms responsible for the familial susceptibility.

A study of 1263 children with acute leukemia at the Children's Cancer Research Foundation in Boston showed the following distribution of cell types: 44% lymphocytic, 25% undifferentiated, 24% myelogenous, and 8% monocytic (4). The lymphocytic excess was limited to a sharp age peak at 2 and 3 years. Myelogenous and undifferentiated categories varied less with age, predominated among males, and had a survival pattern midway between trends for the lymphocytic and monocytic types. The differences between lymphocytic and undifferentiated leukemia were surprising, since these cell types share cytochemical characteristics and are usually considered as one entity. The association of certain cell types with familial clusters and predisposing genetic disorders suggested clues to mechanisms involved in blast-cell differentiation (4).

Newly developed statistical procedures for time-space clustering were applied to the distribution of childhood leukemia in New Zealand, where ascertainment of cases is virtually complete (10). The tendency for cases to cluster was found to be very slight and was attributed to an artefact, namely, the migration patterns of the population at risk.

The possible relation of infectious mononucleosis to leukemia or lymphoma has been questioned with the detection of the EB-virus in patients having mononucleosis or Burkitt's lymphoma, and with reports of concurrent disorders in the same patient. Using data from the National Cooperative Leukemia Survey, in which 12 medical centers participated, mononucleosis was as frequent among leukemic children as among controls, suggesting that mononucleosis neither enhances nor inhibits the development of leukemia (8). In addition, the sibs of leukemic children did not have an unusual frequency of mononucleosis.

Under the auspices of the Commission of Epidemiology, UICC, we began an international survey of childhood cancer (3). The study is based on information routinely collected by tumor registries in various parts of the world. Data have been received now from 20 countries and are expected from several more. Preliminary review of the material according to cell-type classification suggests wide variation in the distribution of certain cancers. Where remarkable differences are found between countries, sections or blocks of the neoplasms will be reviewed under the direction of the project Co-Chairman, Dr. J.N.P. Davies of the Albany Medical College. If geographic concentrations of cancer are confirmed, special investigations will be encouraged to identify etiologic factors.

A peculiar occurrence now under investigation involves an unusually high frequency of acute monomyelogenous leukemia, presenting usually as orbital tumors, in Turkish children. In collaboration with investigators at the Ankara Medical College, we are coordinating etiologic studies on newly diagnosed leukemic children. Sera, skin biopsies, and tumor tissues are shipped for viral, immunological, and cell-transformation studies at NCI laboratories.

Other studies in the field of congenital defects, genetics, and childhood tumors were reported or accepted for publication (6, 7, 9, 15, 22, 27-29, 32). One report traced the events which have resulted in the rising national interest in environmental teratogenesis (19).

Adult Cancer, Not Occupational: Utilizing magnetic tapes provided by the National Center for Health Statistics, a computer analysis was made on data abstracted from the death certificates of the 4-1/2 million persons who died of cancer in the United States, 1950-67. A monograph (2) presents the demographic features of cancer, according to sites specified by the 6th Revision of the International Classification of Diseases. The survey yielded many provocative findings. For example, the non-white population has shown a steady rise over time in cancer rates, which are now 15% higher than those for the white population. The respiratory and upper alimentary tracts were among the sites contributing to the excess risk for Negroes, suggesting unusual exposure or racial susceptibility to carcinogens in the environment. Time trends and geographic variation in the U.S. also make possible the evaluation of suspected carcinogens. For example, following the mass use of cyclamates in 1962 there has been no concomitant change in bladder cancer patterns (1). Recently, the rate of increase for lung cancer in females has risen to surpass that for males; the patterns seem consistent with the later adoption by females of smoking habits, and portend a sharp increase in lung cancer among women over the next few years.

For many cancers, substantial geographic variation in mortality occurred within the United States. Cluster analysis showed that certain neoplasms have remarkable similarities in distribution. Among females, one cluster was represented by cancers of the breast, uterus, ovary, large intestine, and rectum. It is of interest that the same tumors tend to coexist as multiple primary neoplasms in the same person, and as components of a familial cancer syndrome. These cancers appear to share etiologic influences with varying geographic prevalence in this country.

In collaboration with the Johns Hopkins University, we are conducting a cancer mortality survey of diabetics treated at the Joslin Clinic in Boston. The frequency of bladder cancer in recent years is of particular interest, since exposure to cyclamates has been heavier and longer among diabetics than that of the general population. However, preliminary data reveal that the rate of bladder cancer in the past decade is no higher than the frequency of this tumor in the pre-cyclamate era.

Pedigree study and laboratory tests were applied to several family aggregates of cancer (9). We reported a family with ovarian carcinoma in 7, and probably 10 women, in which the distribution suggested a dominant mode of inheritance (12). In family aggregates of leukemia and lymphoma, immunologic abnormalities were detected in leukemic patients and normal relatives, suggesting a familial susceptibility based on genetically-controlled impairment of immune mechanisms. Currently under study is a striking immune disorder in one family: 4 members had lymphosarcoma, 1 had Hodgkin's disease, 1 had Waldenstrom's macroglobulinemia (IgM component), and 3 "normal" members are being followed with hypergammaglobulinemia involving IgM. No consistent defect has been identified as yet in a series of families with multiple cancer syndromes.

A contract was initiated with Harvard University to test the hypothesis that the risk of breast cancer is affected by the relative levels of individual estrogen fractions produced in the first decade after puberty.

In collaboration with the Follow-up Agency of the National Academy of Sciences, which has access to data on the military-veteran population of the U.S. since World War I, data have been collected and analyzed concerning the risk of cancer following selected viral and other conditions diagnosed during military service. A contract with the Agency was expanded to include a new project on Hodgkin's disease, studies of twins with cancer, and preservation of the diagnostic index to the medical experience of the Korean War.

A mortality survey of esophageal cancer in the United States emphasized the susceptibility of Negroes and the relationship to urbanization (25,26).

Under contract with Harvard University, a study was completed on cancer and other causes of death among New England women who received pelvic radiotherapy up to 30 years before. One report presents data indicating that leukemia increases as the radiation dose rises to about 500 rad, and then decreases at higher levels. To explain this paradox it was suggested that local irradiation is leukemogenic when cells are damaged at relatively low doses, but not when they are killed at high doses. A second report identifies a high risk of uterine sarcomas in the study group. During the year, a review of epidemiologic studies on radiation-induced cancer was published (20).

Adult Cancer, Occupational: In collaboration with the National Academy of Sciences, a report was published on the mortality experience of 6,560 males trained as radiological technologists by the U. S. Army during World War II (18). Comparisons were made with men trained as pharmacy or medical laboratory technologists. No form of cancer, including leukemia, occurred excessively. In addition, no disturbance was found in the sex ratio of offspring to suggest a genetic effect of radiation. Another report concerned the relation of lung cancer to occupational exposures to arsenic trioxide and to sulphur dioxide (5).

During the year efforts have been made to develop collaborative studies in the area of occupational cancer. One staff member spent the year at the occupational health field station of the Public Health Service in Salt Lake City, where he supervised a reanalysis of data concerning lung cancer in uranium miners. A monograph on this subject is being prepared.

The Branch continued to encourage establishment of a National Death Index, which would provide a tremendous impetus to occupational studies in cancer (16).

Veterinary Cancer: In 1961 the Epizoology Section was initiated in the expectation that research into neoplasia among domestic animals would provide new insight into the origins of human cancer. At that time, there was an absence of systematically collected data for epidemiologic research. The Section developed a system for making standard observations on a mass scale and to permit machine-processing and retrieval. The system was widely adopted both here and abroad. Virtually all U.S. veterinary medical colleges use it, and half of them send abstracts from each hospital or clinic admission record to our offices in Bethesda (23). Each contributing institution receives a monthly listing of its data; NCI has pooled data for its use. A new coding manual, completed during the year (33), is expected to substantially reduce the time spent by the Epizoology Section in processing the abstracts.

Study of 137,717 patients seen at the participating centers since the beginning of this study revealed 6,455 animals had congenital defects (24). The nature of the malformations differed markedly among the major species. For example, malformations of the eye are more common in the dogs, particularly the Collie, than among other species. Such observations can be of value with regard both to etiology and therapy, since affected animals can be tested in ways that would be inappropriate in man. These studies are being continued with emphasis on the distribution of specific defects.

A file of 8,634 tumors reported to the Section from March 1964 through December 1969 is currently under study. Besides providing an overall description of the occurrence of tumors in domestic animals, it is expected the study will identify high-risk groups which will prove useful in planning studies more directly related to etiology.

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7. Fraumeni, J.F., Jr.: Epidemiology of childhood cancer: Recent developments. Proc. 10th Int. Cancer Congr. In press.

8. Fraumeni, J.F., Jr.: Infectious mononucleosis and acute leukemia. JAMA 215: 1159, 1971.

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10. Glass, A.G., Mantel, N., Gunz, F.W., and Spears, G.F.S.: Time-space clustering of childhood leukemia in New Zealand. J. Nat. Cancer Inst. In press.

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25. Schoenberg, B.S., Bailar, J.C., III, and Fraumeni, J.F., Jr.: Esophageal cancer in the United States: Non-white preponderance and geographic variations in mortality. *Proc. 10th Int. Cancer Cong.* In press.

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Serial No. NCI-4325
1. Epidemiology Branch, OASDD, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Planning and Development in Cancer Epidemiology

Previous Serial Number: Same

Principal Investigator: Robert W. Miller, M.D.

Other Investigators: J.F. Fraumeni, Jr., M.D., W.A. Priester, D.V.M., and other professional staff on the Epidemiology Branch

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.

2. To develop sources of data related to specific epidemiologic problems.

3. To stimulate epidemiologic research in other health agencies.

4. To develop on-the-job training for medical and statistical officers.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.

2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples - clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons (examples - clinical health-surveys, and military medical examinations).

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

To develop opportunities in cancer epidemiology as they arise.

Honors and Awards

Robert W. Miller, M.D.

Speaker

"Relation between Cancer and Congenital Malformations" - Graduate Course in Human Genetics, University of Pennsylvania, September 21, 1970.

"Etiology of Childhood Cancer" - Annual Cancer Retreat, University of Michigan, October 11, 1970.

"Studies in Childhood Cancer as a Guide for Monitoring Congenital Malformations" - Birth Defects Symposium, Albany Medical College, October 19, 1970.

"Relation between Cancer and Congenital Defects" - University of Geneva, Switzerland, November 20, 1970.

"The Link between Congenital Abnormalities and Cancer" - Symposium on Cancer and Other Chronic Diseases in Migrants to Israel, Tel-Aviv, February 22, 1971.

"Genetic and Epidemiologic Aspects of Cancer" - International Symposium on Relationships between Tumor Antigens and Histocompatability Systems, Paris, February 27, 1971.

Society Officer

Council member - The Teratology Society
Council member - Society for Epidemiology Research

Associate Editor

Teratology - Journal of Abnormal Development
Journal of the National Cancer Institute

Committees and Consulting

International Commission on Radiological Protection, Committee 1 on Radiation Effects
National Council on Radiation Protection and Measurements, Committee 32 on Administered Radioactivity
Advisory Committee to the Federal Radiation Council, NAS-NRC--also its Subcommittees on Somatic Effects and on Growth Development
Advisory Committee on the Atomic Bomb Casualty Commission, National Academy of Sciences-National Research Council
Committee on Epidemiology and Veterans Follow-up Studies, National Academy of Sciences-National Research Council
Co-Chairman, Committee on Cancer in Children, Commission on Epidemiology, International Union Against Cancer
Committee on Nutrition, American Academy of Pediatrics
Committee on Environmental Hazards, American Academy of Pediatrics
Committee on Neoplastic Diseases (Consultant), American Academy of Pediatrics
Biometric and Epidemiological Methodology Advisory Committee, Food and Drug Administration
Clinical Professor of Pediatrics, Georgetown University School of Medicine

Visiting Lecturer,
Harvard School of Public Health

Joseph F. Fraumeni, Jr., M.D.

Speaker

"Acute Childhood Leukemia" - 13th International Congress of Hematology, Munich, Germany, August 2, 1970.

"Cancer in Children" - State University of New York at Buffalo, Department of Statistics, Seminar, Buffalo, New York, October 12, 1970.

"Familial Incidence of Cancer" - Johns Hopkins School of Medicine, Oncology Division Conference, Baltimore, Maryland, December 15, 1970.

"Etiology of Leukemia: Epidemiologic Clues" - National Cancer Institute Grand Rounds, Bethesda, Maryland, January 8, 1971.

"Familial Aggregation of Cancer" - Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts, March 16, 1971.

"Etiology of Childhood Cancer: Epidemiologic Clues" - Student Cancer Education Conference, University of Pennsylvania School of Medicine, Philadelphia, Pa., April 17, 1971.

"Epidemiology of Childhood Cancer" - Cancer Day Program, Medical College of Virginia, Richmond, Virginia, April 22, 1971.

Society Officer

President - Assembly of Scientists, National Cancer Institute

Associate Editor

Journal of the National Cancer Institute

Committees and Consulting

Biometry and Epidemiology Contract Review Committee,
National Cancer Institute
Etiology Program Management Group,
National Cancer Institute
Immunology Group, Special Virus Cancer Program,
National Cancer Institute
Breast Cancer Working Group, Special Virus Cancer Program,
National Cancer Institute

Consultant, Advisory Committee on the Biological Effects of Ionizing Radiations, National Research Council - National Academy of Sciences

Fred H. Burbank, M.D.

Speaker

"The Laboratory Computer as a Decisional Instrument" - American Society of Clinical Pathologists, Atlanta, Georgia, September 18, 1970.

Consulting

American Society of Clinical Pathologists, Interim meeting, Las Vegas, Nevada

John J. Mulvihill, M.D.

Speaker

"The Frequency of Congenital Heart Defects in Dogs" - 11th Annual Meeting, Teratology Society, Williamsburg, Virginia, May 3, 1971.

Committees

Consultant, Advisory Committee on the Biological Effects of Ionizing Radiations, National Academy of Sciences - National Research Council

William A. Priester, D.V.M.

Committees and Consulting

Consultant, U.S. Agency for International Development (Uruguay)
Consultant, National Research Council (NAS)
Executive Secretary, Biometry and Epidemiology Contract Review Committee
Committee for Standardization of Nomenclature of Bovine Reproductive Terms (USDA)
Task Force, DHEW, Recommendations Regarding Pending Transfer of DHEW Water Hygiene and Pesticide Laboratories to Environmental Protection Agency

Serial No. NCI-4371
1. Epidemiology Branch, OASDD, Etiology
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: National Cancer Institute Veterinary Medical Data Program

Previous Serial Number: Same

Principal Investigator: W. A. Priester, D.V.M.

Other Investigators: None

Cooperating Units: Schools and Colleges of Veterinary Medicine: Michigan State University, University of Missouri, University of Minnesota, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, Kansas State University, University of Illinois, University of California, Ohio State University, University of Saskatchewan

Man Years:

Total:	2.40
Professional:	.40
Other:	2.00

Project Description

Objectives:

To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed:

Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizoology Section.

Serial No. NCI-4371

Major Findings:

Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from 10 veterinary colleges in this country and 2 in Canada. A modified program has been developed for diagnostic laboratories (pilot study Florida Diagnostic Laboratories). The program has been endorsed and implemented in Europe under the auspices of WHO. For the first time, veterinary medicine and the medical sciences has benefit of a national and international animal disease data system.

Significance to Bio-medical Research and the Program of the Institute:

The comparative (veterinary) medical data retrieval system instituted at the College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to 11 additional veterinary colleges (University of Minnesota, University of Missouri, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, University of California, University of Illinois, Kansas State University, Ohio State University, and the University of Saskatchewan). Establishment and maintenance of reliable sources of veterinary medical data will provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, parasitic diseases, and gerontological diseases. Continued NCI Epidemiology Branch animal studies research function will depend upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease.

Proposed Course of Project:

It is anticipated that this project will continue for a minimum of two years.

Date initiated: October 22, 1965

Current annual level: $21,000

Serial No. NCI-4377
1. Epidemiology Branch, OASDD, Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Familial Aggregation of Malignancies

Previous Serial Number: Same

Principal Investigators: Joseph F. Fraumeni, Jr., M.D. and
Robert W. Miller, M.D.

Other Investigators: John J. Mulvihill, M.D., Robert D. Jensen, M.D.,
Frederick P. Li, M.D., and Miriam D. Manning, M.D.

Cooperating Units: None

Man Years:

Total:	1.10
Professional:	1.00
Other:	0.10

Project Description

Objectives:

To document the occurrence of patterns of family aggregation of neoplasms. To study such families by genetic and laboratory investigations, in an effort to elucidate the degree to which heredity and/or the common familial environment contribute to the etiology of neoplasms. To distribute tissue and blood specimens from such families to interested laboratory investigators, for etiologic study by cytogenetic, immunological, viral, and tissue culture methods. To identify genetic disorders predisposing to cancer.

Methods Employed:

1. Interview of cancer patients with respect to family occurrence of cancer and other disorders, as well as information on prior medical and environmental history.

2. Documentation of history by obtaining appropriate vital records and hospital charts.

3. Collection and distribution of biological specimens from such families.

4. Review of hospital records of series of patients with selected genetic diseases or neoplasms.

5. Survey of childhood cancer death certificates in the U.S. since 1960 to identify sib aggregation.

Major Findings:

Eight non-gonadal tumors were discovered among 289 cases of Turner's (XO) syndrome in four hospitals. These patients and a review of the literature suggest a genetic predisposition for the development of tumors of neural crest origin.

In a study of one family, ovarian carcinoma was diagnosed in 7 women, 4 of whom were sisters, and was suspected in 3 other relatives. Currently under study are familial aggregations of lymphoma, leukemia, male breast cancer sarcomas with other cancers, and medullary thyroid carcinoma with pheochromocytoma.

A survey of mortality records for childhood cancer in the U.S., 1960-67, revealed that presumably identical twins with leukemia had a concordance rate of about 17%. For non-twin sibs, brain tumors were concordant in 8 sib pairs, vs. 0.9 expected. Familial multiple-cancer syndromes were suggested by an excess of sibships with the following combinations of neoplasms: brain tumor with sarcomas of bone or muscle, adrenocortical carcinoma with rhabdomyosarcoma, lymphoma with carcinoma of the colon. The last category included 1 family, studied previously at the NIH Clinical Center with multiple polyposis of the colon and various types of sarcoma (Fraumeni, J.F., et al, Arch. Int. Med. 121: 57-61, 1968).

A survey of 1,263 children with acute leukemia revealed cell-type dissimilarities according to demographic characteristics, familial occurrence, and predisposing heritable conditions, which suggest the influence of genetic factors in blast-cell differentiation.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications, enabling surveillance and early diagnosis of neoplasms.

Proposed Course of Project:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as necessary.

Publications

Wertelecki, W., Fraumeni, J.F., Jr., and Mulvihill, J.J.: Nongonadal Neoplasia in Turner's Syndrome. Cancer 26: 485-488, 1970.

Li, F.P., Rapoport, A.H., Fraumeni, J.F., Jr., and Jensen, R.D.: Familial Ovarian Carcinoma. J.A.M.A. 214: 1559-1561, 1970.

Miller, R.W.: Deaths from Childhood Leukemia and Solid Tumors Among Twins and other Sibs in the U.S., 1960-67. J. Nat. Cancer Inst. 46: 203-209, 1971.

Fraumeni, J.F., Jr., Manning, M.D., and Mitus, W.J.: Acute Childhood Leukemia: Epidemiologic Study by Cell Type of 1,263 Cases at the Children's Cancer Research Foundation in Boston, 1947-65. J. Nat. Cancer Inst. 46: 461-470, 1971.

Serial No. NCI- 4378
1. Epidemiology Branch, OASDD,
   Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: U.S. Cancer Mortality Survey

Previous Serial Number: None

Principal Investigator: Fred Burbank, M.D.

Other Investigators: Joseph F. Fraumeni, Jr., M.D.

Cooperating Units: National Center for Health Statistics

Man Years:

Total:	1.1
Professional:	1.0
Other:	0.1

Project Description

Objectives:

To examine the entire cancer mortality experience in the United States, 1950-1967 for clues to cancer etiology.

Methods Employed:

Computer analysis of over 4.6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated year by year, and expanding. To date, cancer sites have been analyzed by age, time trend, and geographic distribution. In addition, geographic distributions have been analyzed by clustering techniques to gauge which tumors may have a similar etiology.

Major Findings:

For males and nonwhite females cancer is rising in the United States. For white females there has been no change in age adjusted cancer death rates since 1950. Nonwhite male death rates rose higher than white male rates in 1956 and have increased faster than white male rates ever since. Most tumors have higher rates in the Northeastern section of the United States; the rate of increase of all cancer sites is greatest in the South. That is, state cancer death rates are becoming homogeneous throughout the United States.

The sequential geographical cluster--time trend analysis for whites suggests that there are approximately a dozen major carcinogens operating in the United States.

Significance to Bio-medical Research and the Program of the Institute:

This survey investigates a continually expanding data set: U.S. cancer death certificates. Clues to the etiology of cancer can be determined from the pattern of this mortality experience. In addition, specific etiological theories about cancer can be tested against the data set.

Proposed Course of Project:

Continue to add new years to the already existing data, begin to compare to non-cancerous diseases, plan update of Monograph 33 after 1970 census is fully prepared.

Publications

Burbank, F.: Patterns in cancer mortality in the U.S. 1950-1967. National Cancer Institute Monograph 33. 1971, 593 pp. (in press)

Serial No. NCI-4379
1. Epidemiology Branch, OASDD,
   Etiology
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of Congenital Defects in Domestic Animals

Previous Serial Number: None

Principal Investigator: John J. Mulvihill, M.D.

Other Investigators: William A. Priester, D.V.M. and other professional staff in the Epidemiology Branch

Cooperating Units: See Project Report NCI-4371

Man Years:

Total:	0.70
Professional:	0.50
Other:	0.20

Project Description

Objectives:

To study the characteristics of patients with congenital defects for clues as to possible relationships to cancer in the same clinic-hospital population. To relate these findings to human beings.

Methods Employed:

A file of patients with congenital defects, reported to the Veterinary Medical Data Program (NCI-4371) from March 1964, through January 1969, has been related to the population of all patients seen during the same period. Data accumulated since January 1969 can be used to test the conclusions of studies made for the earlier period. General findings have already been published; currently, individual species and diagnoses are under scrutiny, for example, hip dysplasia and patellar dislocation in dogs.

Major Findings:

Preliminary results reported to February 1969 indicated that among 137,717 patients, 6,455 (4.7%) had congenital defects. The frequency was highest in swine and lowest in cats. Some species were prone to specific defects; for example, congenital hip dysplasia developed almost exclusively in dogs, while imperforate anus was very common in pigs. Multiple congenital anomalies were found in 311 animals. Heart malformations occurred in 0.4% of dogs, a figure identical to the frequency in man.

Significance to Bio-medical Research and the Program of the Institute:

Experiments on animals are essential to progress in medical research. Traditionally, a small number of species is used for laboratory experimentation. Currently popular laboratory animals are generally poor models for genetic disease and congenital malformations in man, and the rarity of these individual conditions in the human population limits clinical research. Our project attempts to detect conditions spontaneously occurring in domestic animals which are more nearly homologous to human afflictions. Taking heart malformations in dogs as an example, we have defined high frequencies of 1) patent ductus arteriosus in Miniature-Toy poodles, and 2) persistence of the right aortic arch in German Shepherds. Since certain congenital malformations in man are associated with childhood malignancies, we hope to identify domestic animals which may serve as research models to explore the borderland between teratology and oncology.

Proposed Course of Project:

Data will continue to come from the Veterinary Medical Data Program and will be analyzed for breed-specific lesions.

Publications

Priester, W.A., Glass, A.G., and Waggoner, N.S.: Congenital Defects in Domestic Animals. I. General Considerations. Amer. J. Vet. Res. 31: 1871-1879, 1970.

Mulvihill, J.J. and Priester, W.A.: Frequency of Congenital Heart Defects in Dogs (Abstract). 11th Annual Meeting, Teratology Society, Williamsburg, Virginia, May 1971.

Serial No. NCI - 4380
1. Epidemiology Branch, OASDD, Etiology
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of the zoographic characteristics of domestic animals with tumors.

Previous Serial Number: None

Principal Investigator: W. A. Priester, D.V.M.

Other Investigators: None

Man Years:

Total:	1.40
Professional:	.40
Other:	1.00

Project Description

Objectives:

To provide a reference of the distribution of spontaneous tumors in domestic animals. To search for unusual distribution of tumors, within a large animal hospital population, which might suggest possible clues regarding the etiology of cancer.

Methods Employed:

Using patients with tumors reported to the Veterinary Medical Data Program as case series, and all patients seen during like periods as population references, the following will be studied:

Characteristics of patients: age, breed/species, sex
Characteristics of tumors: degree of malignancy, site, and histogenic type.

In addition, other factors may be studied as the analyses progress.

Serial No. NCI - 4380

Major Findings:

Only preliminary data are available at this time. 8,634 tumors were reported during the period March 1964 through December 1969 among 202,277 patients seen. The proportion of patients with tumors was highest among dogs and lowest among horses, when the four major species categories, bovine, equine, feline, and canine, were considered.

Significance to Bio-medical Research and the Program of the Institute:

Animal studies done by the Branch have demonstrated several relationships of interest in cancer research -- bone sarcoma and size, canine lymphoma and certain breeds, canine mastocytoma and breed ancestry. This study is designed to detect high risk groups in a systematic fashion. The results may well define animal subgroups suitable for studies of cancer etiology that would be impractical in a human population.

Proposed Course of Study:

It is anticipated that the results from the current population under study, 1964-1969, can be used to generate hypotheses that will be tested in the 1970-1971 study population. It is expected the project will continue for a minimum of 3 years.

Date initiated: May 1970

Current annual level: $19,000

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1971

Ecology Studies Section

JOHNS HOPKINS UNIVERSITY (NIH-70-2134)

Title: Epidemiological Study of Cancer Mortality Among Diabetics

Contractor's Project Director: Irving I. Kessler, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To evaluate over time the cancer mortality experience of diabetics, whose *per capita* consumption of cyclamates since 1960 has greatly exceeded that for the general population.

Methods Employed: Utilizing a group of 21,447 diabetics registered at the Joslin Clinic in Boston over a 26-year period, the risk of death from all forms of cancer in the precyclamate and postcyclamate periods will be compared with one another and with the rates prevailing in the general Massachusetts population.

Major Findings: Since 1960, a total of 5 new bladder cancers have been identified, all in males. A total of 141 cancers have been found in males, and 128 in females. Although it appears unlikely that diabetics have a high risk of bladder cancer, it will be important to identify the risks of various cancers in patients with diabetes.

Significance to NCI Program and Biomedical Research: The Epidemiology Branch is concerned with studies relating environmental agents to cancer, and the surveillance of populations exposed to potential carcinogens. A widely used food additive, cyclamates, was found to cause bladder tumors in experimental animals; its carcinogenic potential in man is unknown, but may be clarified by a long-term survey of groups, such as diabetics, with heavier than normal exposure to cyclamates. This study also provides an opportunity to assess the risk of various types of cancer in the diabetic population.

Date Contract Initiated: March 1, 1970

Current Annual Level: $4,955

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, ETIOLOGY
Fiscal Year 1971

Ecology Studies Section

WESTERN RESERVE UNIVERSITY (PH 43-64-524)

Title: Mortality Experience of Children who were Inadvertently Inoculated with SV-40 very Early in Life

Contractor's Project Director: Eli Gold, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To determine whether the inoculation of human infants with polio vaccine containing SV-40 at birth is associated with an increased mortality due to malignant neoplasms.

Methods Employed: The mortality experience of 1,077 mainly Negro children, born in Cleveland, Ohio, between December 1959 and May 1962 and inoculated with SV-40 shortly after birth, will be followed through time. The location and name of each child are determined annually. Deaths are discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The names in the study group are compared against an alphabetic file of all childhood cancer deaths in the U.S. maintained in the Epidemiology Branch, NCI. The frequency of cancer deaths in the study group will be compared with the expected cancer mortality for children in the State of Ohio.

Major Findings: As yet there have been no cancers or other disorders in the study group attributed to SV-40. An interim report of the findings was published in Science 167: 59-60, 1970.

Significance to NCI Program and Bio-medical Research: Previous investigations by the Epidemiology Branch of school-age children who were inadvertently inoculated with SV-40 will be extended. The known importance of neonatal exposure in the causation of many viral neoplasms of animals suggests the possibility that the cancer mortality of children inoculated at birth might be different from that of children exposed at school age. The present study will extend our present knowledge in this area.

Proposed Course of Project: Since neoplasms with a latent period longer than 8 years could not be detected by the current follow-up, surveillance of the children will continue to be maintained.

Date Contract Initiated: March 9, 1964

Current Annual Level: $9,251

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)

Animal Studies

Personal Service Contracts between the Epizootiology Section and Colleges of Veterinary Medicine at the University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, Kansas State University, Ohio State University, University of Saskatchewan, Purdue University, University of Minnesota, and University of California.

Title: Purchase Veterinary Case Data.

Objectives: To add to volume of stored data, available to Epizootiology Section, in the Veterinary Medical Data Program. To further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: Minimum of two years.

Dates Initiated:
Michigan State University - November 1965
Iowa State University - October 1965
Ontario Veterinary College - July 1966
University of Georgia - January 1967
University of Missouri - February 1967
University of Minnesota - December 1967
University of Illinois - January 1968
Kansas State University - January 1968
Purdue University - January 1968
Ohio State University - January 1969
University of Saskatchewan - January 1969
University of California - July 1970

Current Annual Level: $30,000

Summary Report
Epidemiologic Pathology Unit-Etiology Area
July 1, 1970 through June 30, 1971

With the failure to obtain a third pathologist who would have expanded and fully developed our projects in quantitative pathology, it was decided to abandon this line of investigation. Instead, the primary task will be development of interdisciplinary programs uniting and expanding Etiology's laboratory and epidemiological investigations of particular kinds of cancer. The first of the year an Ad Hoc Committee, chaired by Dr. Berg has been formed to assist in review and development of Etiology programs relating to colon cancer carcinogenesis. This committee will be complimentary to another being formed by Dr. Berg to develop and presumably operate a segment of the National Program on Colon-Rectum Cancer. It is hoped to maintain in the Unit the current balance between administrative efforts and actual analyses needed for program development.

Consultative work has continued particularly in regard to the Migrant Program, the epidemiologic aspects of the Carcinogenesis contract programs, the Third National Cancer Survey and outside committees as described below. Dr. Lingeman and Mr. Feldman have pursued their respective interests as evidenced by their project reports and bibliographies.

Serial No. NCI 4300
1. Epidemiologic Pathology Unit,
OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mathematical Theory of Complex Ligand-Binding Systems

Previous Serial Number: New

Principal Investigator: Henry Feldman

Other investigators: David Rodbard, Reproduction Research Branch, NICHD (formerly Endocrinology Branch, NCI)

Cooperating Unit: Mathematical Statistics and Applied Mathematics Section, Demography, NCI and Program Analysis Branch, Chemotherapy, NCI

Man Years:

Total:	1.4
Professional:	1.3
Other:	0.1

Project Description

Objectives:

To provide theoretical basis for the understanding of certain naturally occurring and widely-used chemical reactions.

Methods Employed:

Theory of applied mathematics, electronic computation, computer-linked plotting machine.

Major Findings:

Mathematical theory and computational methods have been developed for the study of biochemical systems in which one or more ligands (antigens, hormones, ions) react simultaneously and independently with one or more binding molecules (antibodies, receptors, enzymes). The theory pertains directly to radioimmunoassay and radio-ligand assays and represents a broad extension of classical theory in these fields. In particular: (1) Equations have been developed which characterize the equilibrium composition of the reacting system. Numerical methods have been developed for solving these equations by computer. By this method large numbers of simulated assays have been conducted with systematic variation of assay parameters, such as affinity constants and antibody concentrations. The results of these assays have been depicted in a compact graphical format by a computer-linked plotting machine. Inspection of the graphs has led to several conclusions regarding optimal conditions for sensitivity and precision of assays. The technique of simulation promises to provide a powerful method for testing hypotheses regarding many biochemical systems. (2) The same equations have been utilized in fitting parameters (affinity constants, reactant concentrations) to experimental data on multi-component ligand-binding systems. The computations have again been programmed for computer, and, when implemented on a simple, laboratory-available basis, should prove a valuable tool both in characterizing new ligands and in detecting, identifying and measuring old ones. In addition to the publications listed, four other papers have been prepared and submitted for possible publication.

Significance of the Program to the Institute:

Many studies are using and even more will be using radioimmunoassays. This work shows how more information can be generated more quickly and more reliably and permits more accuracy in the identification of known compounds and the characterization of new ones.

Proposed Course of Project:

Dr. Rodbard will continue the use of the simulation and parameter-fitting tools and will extend research into statistical questions. Mr. Feldman will be extending this line of inquiry in the doctoral program of Harvard University.

Publications

Feldman, H., Levine, D., and Rodbard, D.: Theory of complex ligand-binding systems: Applications to radioimmunoassay and plasma steroid-protein equilibria. Proceedings, 10a Reunion Anual, Sociedad Mexicana de Endocrinologia y Nutricion, pp. 615-632, 1970.

Feldman, H., and Rodbard, D.: Mathematical theory of radioimmunoassay. To be published in a volume resulting from the Endocrine Society's Training Course in radioimmunoassay, Harbor General Hospital, Torrance, California, March 29-April 2, 1971.

Feldman, H., and Rodbard, D.: Multi-component ligand-binding systems: Computer simulation, parameter-fitting, and a generalized scatchard plot. Federation Proceedings Abstract Supplement, May-June 1971.

Rodbard, D., Feldman, H., and Levine, D.: Mathematics and statistics of radioimmunoassays (abstract). Biometrics 27: 259, 1971.

Serial No. 4397
1. Epidemiologic Pathology Unit, OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Comparative Pathology of Malignant Neoplasms of Animals and Man

Previous Serial Number: Same

Principal Investigator: Carolyn H. Lingeman

Other Investigators: None

Cooperating Units: Epidemiology Branch, National Cancer Institute, Bethesda, Maryland; Veterinary Pathology Section, Armed Forces Institute of Pathology, Washington, D.C.

Man Years:

Total:	1.2
Professional:	1.0

Project Description

Objectives:

To compare clinical, epidemiologic, and morphologic features of spontaneous and experimental malignant neoplasms of animals and man to attempt to relate the factors involved in their etiologies to the human counterparts.

Methods Employed:

1. In cooperation with veterinary pathologists of the Comparative Pathology Branch of the Armed Forces Institute of Pathology, the California Animal Neoplasm Registry, and other institutions, to investigate systematically the epidemiologic, clinical, and morphologic characteristics of prevalent neoplasms of man that have morphologic counterparts in animals:

    a. Hematopoietic neoplasms
    b. Gastric adenocarcinomas
    c. Intestinal adenocarcinomas
    d. Mammary gland carcinomas

e. Others to be selected, from among tumors of endocrine organs, liver, pancreas, connective tissue, lung ,etc.

Major Findings:

1. Hematopoietic neoplasms: General results were summarized in NCI Monograph #32 and annual reports of previous years. Studies on specific topics such as mast cell neoplasms and thymomas of cats are continuing.

2. Gastric adenocarcinomas: These neoplasms are extremely infrequent in animals and are induced by chemical carcinogens only with extreme difficulty. Dogs appear to develop spontaneous gastric adenocarcinomas most frequently. The canine neoplasm is similar to the diffuse histologic type of man. As in man, most arise in the antral segment, along the lesser curvature and metastasize to lymph nodes and liver. They occur in older dogs of both sexes and there is no breed preference.

3. Intestinal adenocarcinomas are also infrequent in animals compared to man. They are most frequent in dogs and hamsters. The canine carcinomas may arise in polypoid adenomas, and may metastasize to lymph nodes and liver. They occur in older dogs and no sex or breed preference has been noted.

   Contrary to the distribution in man, carcinomas of the small intestine are more frequent than those of colon and rectum in cats, sheep and some other animals. The distribution of induced tumors depends on the agent and means of induction. The basic manuscript on this topic in comparative pathology has been prepared and is now being reviewed prior to publication.

4. Mammary gland carcinomas are among the most frequent neoplasms in many mammals including rats, mice, cats and dogs. They are apparently rare in other rodents, cattle, horses, sheep, pigs and primates.

   The murine carcinomas have been well-characterized. The canine neoplasms are a complex group of benign and malignant growths involving both epithelial and connective tissues. They are hormone-dependent.

   The feline neoplasms are mostly adenocarcinomas and are morphologically similar to some human types. There is a need to characterize the clinical, epidemiologic, and morphologic characteristics of the feline neoplasms.

Serial No. NCI 4397

Significance of the Program to the Institute:

Pathologists must provide support for epidemiologic and experimental investigations of neoplasms by providing a sound morphologic basis in terms which are consistent and applicable to a wide range of species of animals. Nomenclature and systems of classification are basic to this task.

Proposed Course of Project:

1. To continue to investigate comparative aspects of human neoplasms and their animal counterparts in order to discover factors that may be involved in their etiologies.

2. To suggest animal models for experimental testing.

Publications

Lingeman, C. H., Garner, F. M., and Taylor, D. O. N.: Spontaneous gastric adenocarcinomas of dogs. A review. J. Nat. Cancer Inst. (In press).

Garner, F. M., and Lingeman, C. H.: Mast cell neoplasms of the domestic cat. Pathologia Veterinaria (In press).

Serial No. NCI 4398
1. Epidemiologic Pathology Unit,
OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Registry Based Pathology Studies of Cancer

Previous Serial Number: Same

Principal Investigator: John W. Berg

Other Investigators: Henry Feldman, Andrew Huvos*, Steven I. Hajdu*, Frank W. Foote, Jr.*, Robert V. P. Hutter*, Guy F. Robbins*, Basil Morson**

Cooperating Units: Departments of Pathology, Surgery, Memorial Hospital for Cancer and Allied Disease, New York City*; End Results Section, National Cancer Institute; Connecticut Cancer Registry; St. Mark's Hospital, London, England**; Migrant Studies Program, Biometry Branch, National Cancer Institute

Man Years:

Total:	1.6
Professional:	0.9
Other:	0.7

Project Description

Objectives:

1. To analyze pathology data now existing in tumor registries by study of epidemiology and survival correlations.

2. To utilize existing registry and related data in the solution of specific pathology problems such as the significance of specific histologic patterns and the behavior of rare tumors.

3. To discover what further pathology data are needed and could be used in epidemiological and end-result studies and which types of Registry data are particularly needed to help solve problems in epidemiological pathology.

4. To plan for and collect such data in a form suitable for modern types of computer-based statistical analysis.

5. To undertake studies in quantitative cancer pathology and to base as many of these as possible on Registry-defined data.

Methods Employed:

Computer surveys of large record files, record review of specific sets of cases, statistical analyses, and qualitative and quantitative microscopic studies of particular pathology material all are used when appropriate.

Major Findings:

1. Studies on nomenclature and classification of tumors have been expanded. A second edition of the Systematized Nomenclature of Pathology of the College of American Pathologists is being prepared. Collaboration with the National Office of Health Statistics continues in preparing recommendations for the forthcoming 9th Revision of the International Classification of Diseases. The 1968 Edition of the American Cancer Society's Manual of Tumor Nomenclature and Coding has been published and studies on the conversion of cancer registry data to this more powerful coding scheme are continuing.

2. The importance of the amount of metastatic cancer present in lymph nodes has been shown by relating quantity of tumor to survival. A study of the relation of the amount of benign lymphoid tissue in nodes to survival is now in progress.

3. Epidemiological-pathological studies of specific types of cancer are continuing. Studies on lung cancer not only led to a report but formed the scientific basis for work done as NCI representative on committees dealing with lung cancer in uranium miners and smoking dogs. In collaboration with the WHO Reference Center for Intestinal Tumors bowel cancers were analyzed from this standpoint using histological material from the Migrant Studies, unpublished Registry data from Connecticut, Memorial Hospital for Cancer and Allied Diseases, and Sendai, Japan as well as published data.

4. The most recent incidence data from the Connecticut Cancer Registry (through 1966) was surveyed for unusual changes in the frequency of appearance of specific tumor types. In addition to already recognized increases in such general categories as bladder cancer, 9 specific histologic types of cancer were found to be occurring in increased numbers. This information was reported to Connecticut for investigation.

Significance of the Program to the Institute:

While individual studies have intrinsic value, the prime aim of the project is to provide a broad factual framework for use in planning the programs of the Etiology Area. In particular it aims for a more specific identification of the heterogenous assortment of diseases including "cancer" of the various anatomic sites and for development of more precise and rigorous techniques for quantitative description of human cancers.

Proposed Course of Project:

As studies now underway are completed, others will be added in the light of the concerns of the Etiology Area and the Unit's capacity. In addition we hope that we can facilitate greater use by others, both in and outside Etiology, of the ever increasing number of analyses we have made from what surely must be the largest cancer registry oriented pathology data bank (or pathologically oriented cancer Registry data bank) in existence. One collaborative study with the End Results Group data has been completed, but unfortunately, up to this time it has not been possible for the Unit to participate in any publications of the Connecticut Cancer Registry or the Migrant Program. Although the way also has been opened for international work in comparative epidemiology and pathology, the present limitations on foreign collaboration make substantive progress very slow and uncertain.

Honors and Awards

Member, HEW Health Options Working Group; Member, Interagency Uranium Miners Review Group Subgroup IVB; Chairman, Ad Hoc Carcinogenesis Committee on Colon Cancer; Member, Planning Group, National Program for Colon Cancer; Member, Task Forces on Lung Cancer, Melanoma and Breast Cancer, American Joint Committee on Cancer Staging and End Result Reporting; Chairman, Subcommittee on Morphology, Committee on Nomenclature and Classification of Disease, College of American Pathologists; Member, Task Force to Revise "Manual of Tumor Nomenclature," American Cancer Society; Consultant in Epidemiologic Pathology, Memorial Hospital for Cancer and Allied Diseases; Member, Clinical Investigation Advisory Committee, American Cancer Society; Member, Lung Cancer Pathology Advisory Committee, American Cancer Society; Member, Working Party on Classification of Neoplasms of the Revision of the ICD, U.S. National Committee on Vital and Health Statistics.

Publications

Berg, J. W.: Some intercountry and intergroup differences in histological types of cancer. J. Chron. Dis. 23: 325-334, 1970.

Berg, J. W.: Epidemiology of the different histologic types of lung cancer. In "Morphology of Experimental Respiratory Carcinogenesis," AEC Symposium Series 21: 93-104, 1970.

Serial No. NCI 4398

Huvos, A. G., Hutter, R. V. P., and Berg, J. W.: Significance of axillary macrometastases and micrometastases in mammary cancer. Ann. of Surg. 173: 44-46, 1971.

Serial No. NCI 4399
1. Epidemiologic Pathology Unit,
OASDD, ET
2. Office of the Head
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Incidence Studies of Multiple Primary Cancers

Previous Serial Number: Same

Principal Investigator: John W. Berg

Other Investigators (Not NCI): David Schottenfeld*, Steven Hajdu*, Frank W. Foote, Jr.*

Cooperating Institution: *Memorial Hospital for Cancer and Allied Diseases, New York City

Man Years:

Total:	.5
Professional:	.3
Other:	.2

Project Description

Objectives:

To analyze rigorously the true incidence of subsequently developing primary cancers in populations of cancer patients and thereby identify high risk situations and epidemiologically and etiologically important patients subsets.

Methods Employed:

The man years of observation for patients divided by age and sex are calculated for the entire Memorial Registry population of 50,000 using the computer facility of the Department of Medical Physics, Memorial Hospital. The charts of patients with more than one cancer are then studied in detail. Pathologic material from the cases is reviewed to clarify controversial situations. Detailed investigations of high risk situations are undertaken by David Schottenfeld. Separate analyses have been made of latent cancer found at autopsy.

Major Findings:

In the study as a whole, 793 cancers were expected in organ systems other than that of the first cancer. Twice as many, 1643, were found. Part of the increase seems to be procedural due to earlier detection. Most of the remaining cancer excess is a very specific susceptibility of related epithelia or of exposure of several organs to a single carcinogen such as tobacco. Few cancer patients, if any, in our study had a general susceptibility nor was there evidence of a polyoma virus-like effect with frequent appearance of tumors either clinical or subclinical in multiple tissues.

Significance of the Program to the Institute:

The Memorial case file with its high concentration and specific follow-up represented the best material in existence for this type of study. The material was readily accessible, particularly adequate for pathological review. It can be studied in a way fully compatible with similar analyses which we and others have done on the data of the Connecticut Cancer Registry. The results also link with the study of cancer associations of Dr. Burbank, Epidemiology Branch, NCI.

Proposed Course of Project:

The survey and related calculations were completed in fiscal '68. Studies of high risk situations are continuing as detailed analyses are prepared for publication. An intensive study of factors relating to the risk of multiple colon cancers is beginning.

Publications

Berg, J. W., Hajdu, S. I., and Foote, F. W., Jr.: The prevalence of latent cancers in cancer patients. Arch. Path. 91: 183-186, 1971.

Hajdu, S. I., Berg, J. W., and Foote, F. W., Jr.: Clinically unrecognized, silent renal-cell carcinoma in elderly cancer patients. J. of the Amer. Geriatrics Soc. 18: 443-449, 1970.

Schottenfeld, D., and Berg, J. W.: Incidence of multiple primary cancers. IV. Cancers of the female breast and genital organs. J. Nat. Cancer Inst. 46: 161-170, 1971.

SUMMARY STATEMENT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

July 1, 1970 through June 30, 1971

Umberto Saffiotti, M.D.

The Annual Report for Carcinogenesis, Etiology, NCI, is presented in the following sequence:

I. *CARCINOGENESIS PROGRAM*

- A. *INTRODUCTION*
- B. *PROGRAM PLANS*
- C. *MANAGEMENT ACTIVITIES*
- D. *SCIENTIFIC ACTIVITIES: SUMMARY REPORT AND PROGRESS HIGHLIGHTS*
  - 1. Program Planning
  - 2. Information Systems
  - 3. Resources
  - 4. Carcinogenesis Bioassays
  - 5. Development of Biological Models
  - 6. Identification of Carcinogenesis Processes

II. *OFFICE AND BRANCH REPORTS*

- A. *OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR*
  - 1. Summary Report
  - 2. Individual Project Reports
- B. *BIOLOGY BRANCH*
  - 1. Summary Report
  - 2. Individual Project Reports
- C. *CHEMISTRY BRANCH*
  - 1. Summary Report
  - 2. Individual Project Reports
- D. *EXPERIMENTAL PATHOLOGY BRANCH*
  - 1. Summary Report
  - 2. Individual Project Reports

III. *CONTRACT NARRATIVES*

- A. *INDIVIDUAL CONTRACT NARRATIVES*
- B. *LIST OF CONTRACTS IN PROCESS OF NEGOTIATION*

I. *CARCINOGENESIS PROGRAM*

A. *INTRODUCTION*

The Carcinogenesis Area is responsible for planning, implementing, and managing the National Cancer Institute's coordinated research program on carcinogenesis by chemical and physical factors and on its prevention. In order to meet this responsibility, three distinct program approaches have been established:

(1) Identification of carcinogenic activity of selected agents by *bioassays*;

(2) Development and selection of *biological models* for bioassay and for the characterization of carcinogenesis processes; and

(3) Identification of *processes* required for carcinogenic action of selected agents as target points for corrective measures.

The Carcinogenesis Program consists of an *intramural* component devoted primarily to basic and applied research and an *extramural* component devoted primarily to directed targeted research through the contract mechanism. There is no clear cut programmatic distinction between the two except from an administrative standpoint. They are, in fact, designed to complement each other.

Historically, this Program developed from the Carcinogenesis Studies Branch established in March 1961 as part of the Field Studies Area, for intramural and collaborative investigations on carcinogenic factors, particularly as hazards in the human environment. With the reorganization of NCI and the establishment of the Office of the Scientific Director for Etiology in January 1966, the Carcinogenesis Area was organized under the Office of the Associate Scientific Director for Carcinogenesis (OASDC), with the task of "planning and administering a program of basic and applied research in carcinogenesis leading to the identification or definition of environmental carcinogens, and to the elucidation of carcinogenesis mechanisms." A Biology Branch and Chemistry Branch were established to implement this program.

A contract-supported collaborative program was developed since 1962 to respond to the need for extensive bioassay testing and screening of potential environmental chemical carcinogenic hazards, and for a variety of fundamental research programs on mechanisms of carcinogenesis.

The scope of the whole Carcinogenesis Program was reassessed in 1968. A Program Plan on Chemical Carcinogenesis and Prevention of Cancers was designed and approved: new directions were established for both intramural and collaborative activities.

In 1968-1969 the intramural laboratories and offices were moved from temporary quarters to the new Building 37 on the NIH reservation. Unfortunately, at that time, when a major expansion in the programs was called for, the Carcinogenesis Area was hit by the reduction in positions that affected all Institutes. The total position strength underwent a loss of about 20%. Only now, at the end of Fiscal Year 1971, has the position ceiling reached back at the 1968 level. During these three years, the Carcinogenesis staff gave invaluable cooperation to the task of not only maintaining but also substantially expanding the Carcinogenesis Program activities, both in-house and through contracts, at a time when a rapidly growing public awareness of the problems of environmental cancer hazards was giving a clear indication to the Nation of the increasing needs in this field.

In spite of this period of "depression" in positions, by reassigning space, personnel and individual assignments, and above all by the untiring efforts and cooperation of many members of the staff, several major new programs were activated.

A new Experimental Pathology Branch was established besides the two previously existing ones: Biology Branch and Chemistry Branch. Three special Units were set up in the Office of the Associate Scientific Director: an Epidemiologic Pathology Unit (recently transferred from the Demography Area), a Lung Cancer Unit, and a Program and Data Analysis Unit.

The organization of the Carcinogenesis Area is therefore presently divided into the following groups: The Office of the Associate Scientific Director with three special Units, the Biology Branch, the Chemistry Branch, and the Experimental Pathology Branch, as shown in the attached chart.

The present organization include the following components:

> *The Office of the Associate Scientific Director for Carcinogenesis* (U. Saffiotti, M.D., ASD) includes three units: Epidemiologic Pathology Unit (J. Berg, M.D., Head), Lung Cancer Unit (M. B. Sporn, M.D., Head), and Program and Data Analysis Unit (J. A. Cooper, II, Ph.D., Head).
>
> *The Biology Branch* (H. J. Rapp, Sc.D., Chief) includes three sections: Cellular Immunity Section (B. Zbar, M.D., Head), Cytogenetics and Cytology Section (J. A. DiPaolo, Ph.D., Head), and Immunochemistry Section (T. Borsos, Sc.D., Head); and one unit: Molecular Separation Unit (H. J. Rapp, Sc.D. Acting Head).
>
> *The Chemistry Branch* (H. V. Gelboin, Ph.D., Chief) includes four sections: Cell Growth Regulation Section (J. P. Bader, Ph.D., Head), Molecular Carcinogenesis Section (H. V. Gelboin, Ph.D., Acting Head), Nucleic Acids Section (C. W. Dingman, M.D., Head), and Protein Section (A. C. Peacock, Ph.D., Head).

The Experimental Pathology Branch (R. R. Bates, M.D., Acting Chief) includes two sections: Bioassay Section (R. R. Bates, M.D., Head) and Carcinogen Screening Section (J. H. Weisburger, Ph.D., Head); and one unit: Histopathology Unit (R. R. Bates, M.D., Acting Head--Formerly M. D. Reuber, M.D., Head).

The collaborative contract-supported program was developed into the following five major segments of operation, now actively under way:

(1) *Bioassays,* with emphasis on the following categories of compounds: (a) pesticides and agricultural chemicals; (b) pharmaceutical agents including cancer chemotherapeutic agents; (c) industrial chemicals of several types; (d) food constituents, additives, and contaminants; (e) natural products, particularly mycotoxins; (f) metals and metallo-organic compounds; (g) air pollutants, including synthetic smog, asbestos, metal oxides, and salts; and (h) tobacco and related products.

(2) *Biological Models* for the induction and control of the main types of cancer as seen in man, with emphasis so far on (a) respiratory tract cancers; (b) gastro-intestinal tract cancers; (c) bladder cancers; (d) leukemias and lymphomas; (e) skin cancer; (f) liver cancers; (g) childhood cancers; (h) endocrine related cancers; (i) the development of short-term bioassays using both *in vivo* and *in vitro* systems.

(3) *Chemistry and Molecular Carcinogenesis,* which supports studies aimed at understanding and defining key biochemical processes in cell growth control and in the interaction of carcinogens with cell constituents, that are potentially exploitable targets for the inhibition of carcinogenesis, with emphasis on (a) enzymatic control mechanisms of the activation and metabolism of chemical carcinogens; (b) vertebrate somatic cell genetics; (c) role of nucleic acid metabolism and particularly role of RNA repair mechanisms in carcinogenesis and role of RNA in the control of carcinogenesis; (d) role of protein synthesis control systems, and role of membranes in the control of penetration and interaction of carcinogens in their target cells.

(4) *Biology and Immunology,* which supports studies designed to develop and apply immunological tools for the detection of the effects of carcinogens and for the definition of the immunological factors that control the induction of tumors and particularly the host response. Carcinogenesis at the cellular level is studied by *in vitro* transformation systems.

(5) *Resources and Information,* which supports resources for the entire program, with emphasis on the following: (a) centralized supply of standardized samples of chemical carcinogens and related compounds; (b) analytical chemical resources; (c) preparative chemical resources required to prepare specialized

materials for bioassay; (d) animal resources to supply standardized animal colonies; (e) information resources on the carcinogenesis literature and on all published carcinogenesis tests; (f) computerized data systems for the retrieval and analysis of bioassay data; (g) development of a selection and ranking system for priorities for carcinogenesis bioassays.

A special appropriation, first made in FY 1969 for the Lung Cancer Task Force, was devoted to establish a coordinated program on tobacco carcinogenesis aimed at defining a "less hazardous cigarette." The Tobacco Working Group, established at NCI in 1968, served as the advisory body for the development of this program, which was set up to include bioassays of cigarette smoke of different types in a battery of bioassay systems, side by side with studies aimed at improving and defining biological models for respiratory carcinogenesis. Since this special appropriation did not increase in subsequent years, the Carcinogenesis Program supported an increasing portion of this work under its general appropriation. In FY 1971 the special appropriation from the Lung Cancer Task Force has been used in its entirety to cover the tobacco projects.

The complex operation of the intramural activities and of the collaborative research, supported by contracts in Carcinogenesis, requires the development of a more effective and articulated management structure. The position of Coordinator of Collaborative Research has been established and filled by an experienced scientist manager, Dr. Allen H. Heim. Other staff assignments are presently being considered for several science managers to be assigned to the various contract programs. The major segments of the collaborative programs now underway or being developed will thus be assigned to the scientific supervision of groups of staff members, particularly competent in the different areas of the program.

The Carcinogenesis Intramural Program Group, formally established in this fiscal year, provides a coordinating and planning function at the senior staff level. The entire professional staff is now essentially participating in the planning and reviewing of the overall program, providing a vital correlation between intramural and collaborative research programs.

B. *PROGRAM PLANS*

The overall plan for the Carcinogenesis Program was laid down in 1968 and has served as a solid basis for the implementation of new efforts, as a guide for the expansion of the Program and as a reference for its monitoring. Particular emphasis is now given to the development of special programs on the main organ-specific types of cancers, as seen in man. The first of these to be implemented was the Special Lung Cancer Program, developed in FY 1969, partly by the Lung Cancer Task Force and by the Tobacco Working Group. Its general structure was patterned on that of the general Carcinogenesis Plan. An intramural Lung Cancer Unit was established to serve as a research focus in this effort and to provide strong correlation between staff and contractors.

A new plan is now being developed on the etiology of colon cancer, through an ad hoc group chaired by Dr. J. W. Berg, with the collaboration of Dr. J. H. Weisburger, and other staff members: their direct research on colon cancer pathogenesis represents some of the most advanced experience in epidemiologic pathology and carcinogenesis mechanisms in this special field. A contract program has been initiated in this important area, according to the new plan.

A preliminary exploratory documentation effort on endocrine-related cancers has been initiated by Dr. R. Bates to provide a basis for the future establishment of a special program in this field; the Endocrine Evaluation Branch, GL&C, has accepted to collaborate in this phase.

Preliminary steps have been taken towards the development of special programs on childhood cancers, on bladder cancer and on cancer of the pancreas.

In response to the new proposals for the establishment of a National Cancer Conquest Program, involving much higher funding levels, the Carcinogenesis staff has prepared an outline of a five-year plan for an expanded coordinated program in chemical carcinogenesis and cancer prevention, and a number of supporting documents.

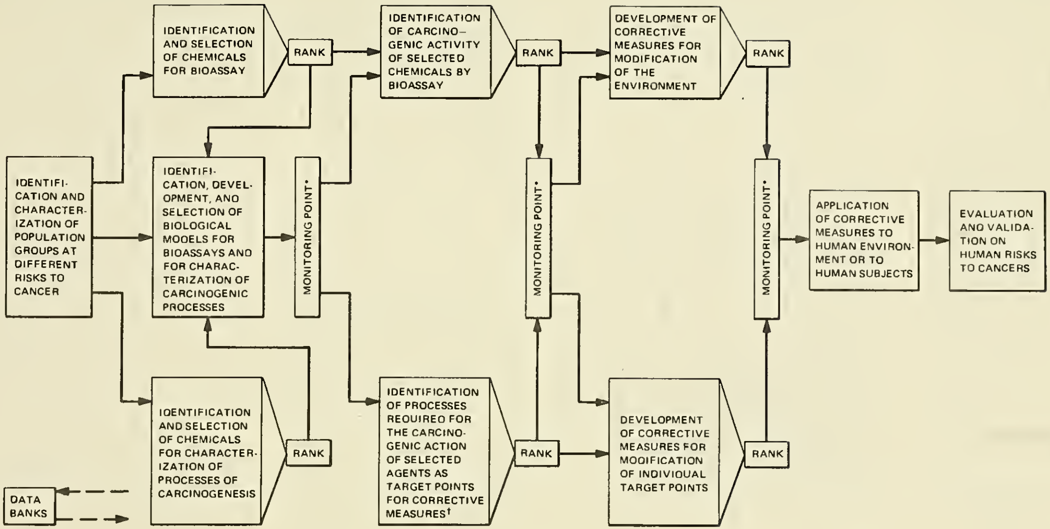
A summary of the general Carcinogenesis Plan, of the Special Lung Cancer Program, and of the preliminary outline of a Five-Year Plan are given below.

1. *Program Plan on Chemical Carcinogenesis and Prevention of Cancers*

The plan was developed in 1968 through an extensive series of planning sessions conducted by Dr. Carl G. Baker, then Scientific Director for Etiology, Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, and Mr. Louis M. Carrese, with the participation of Dr. Abraham Cantarow, and Mr. Richard A. Terselic. The Report of the Discussion Groups on Chemical Carcinogenesis, coordinated by Dr. Cantarow for the National Advisory Cancer Council, served as an excellent basis for outlining the research needs in the field of carcinogenesis. The convergence technique was used to identify the major objectives and the linear array of subsequent phases leading to their implementation. An outline of the program plan is attached and its main phases are discussed below. The program is articulated in a number of segments. The first segment deals with (a) the epidemiologic identification of population groups showing different risks to different types of cancer and (b) to the characterization of such individuals as to their environmental factors and their biological and functional parameters. The program then develops the following three distinct approaches:

(a) identification of carcinogenic activity of selected chemicals by bioassay,

(b) identification, development, and selection of biological models for carcinogenesis bioassays as well as for the characterization of carcinogenesis processes,


IDENTIFICATION AND CHARACTERIZATION OF POPULATION GROUPS AT DIFFERENT RISKS TO CANCER
IDENTIFICATION AND SELECTION OF CHEMICALS FOR BIOASSAY
IDENTIFICATION, DEVELOPMENT, AND SELECTION OF BIOLOGICAL MODELS FOR BIOASSAYS AND FOR CHARACTERIZATION OF CARCINOGENIC PROCESSES
IDENTIFICATION AND SELECTION OF CHEMICALS FOR CHARACTERIZATION OF PROCESSES OF CARCINOGENESIS
RANK
RANK
MONITORING POINT*
IDENTIFICATION OF CARCINOGENIC ACTIVITY OF SELECTED CHEMICALS BY BIOASSAY
IDENTIFICATION OF PROCESSES REQUIRED FOR THE CARCINOGENIC ACTION OF SELECTED AGENTS AS TARGET POINTS FOR CORRECTIVE MEASURES†
RANK
RANK
MONITORING POINT*
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF THE ENVIRONMENT
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF INDIVIDUAL TARGET POINTS
RANK
RANK
MONITORING POINT*
APPLICATION OF CORRECTIVE MEASURES TO HUMAN ENVIRONMENT OR TO HUMAN SUBJECTS
EVALUATION AND VALIDATION ON HUMAN RISKS TO CANCERS
DATA BANKS

*Monitoring points: According to program relevance and needs, determine further action and channel through next phases.

†Through studies on the penetration of the agents into the organism; their transport, retention, and elimination; their metabolic conversion to the proximate carcinogen, the cellular and tissue factors required for such processes; the penetration into target cells and interaction with cell constituents, the neoplastic transformation; and the factors required for the growth of transformed cells into tumors.

CHEMICAL CARCINOGENESIS AND PREVENTION OF CANCER (SUMMARY OF PROGRAM PLANS).

(c) identification of processes required for the carcinogenic action of selected agents as target points for corrective measures.

The first approach requires an initial phase devoted to the identification and selection of chemical agents to be entered in the bioassay systems. The development and implementation of bioassay procedures will require a great deal of accurate standardization so that selected chemical agents will be tested through a battery of precisely defined and reproducible bioassay systems, each well characterized for its sensitivity to specific carcinogenic effects.

After appropriate monitoring for relevance and for program needs, the results of the first phases of the program will lead to the development of corrective measures based, on one hand, on modifications of the environment and, on the other hand, based on modifications of individual target points in the process of carcinogenesis, identified by previous studies. These target points can be visualized as taking place at any one of the several steps required for the process of carcinogenesis to be completed. They include the following: (a) penetration of the chemical agent into the whole organism; (b) transport, retention and elimination of the chemicals in tissues (molecular logistics); (c) metabolic pathways to proximate carcinogen; (d) cell and tissue factors required for the previous steps; (e) penetration into target cells and interaction with cell constituents; (f) neoplastic transformation and conditioning factors; and (g) growth regulation of transformed cells.

The results thus obtained will be in turn appropriately monitored for relevance; the program then considers the development of corrective measures and ultimately suggests application of such corrective measures through the human environment or through the human subject. The last phase of the program deals with the evaluation and validation of the results of such corrective measures in the human situation and is expected to lead to the recognition of decreased cancer risks and in the populations originally studied in Phase I. Data banks will be organized for each of the major phases of the program and they will be a necessary source of information to be used for the monitoring of the whole program.

The program is aimed at a comprehensive attack to the problem of chemical carcinogenesis. It recognizes the need for very extensive bioassay efforts of an order of magnitude much greater than that presently available, but also recognizes that the accumulation of information on the carcinogenicity of chemicals in animal systems will not provide a direct extrapolation to the hazards in man. Moreover, it is predictable that a large number of chemical carcinogens will be difficult to remove and, therefore, will remain in the environment of man for a long time to come even after being identified (e.g. combustion products, cigarette smoke, natural food contaminants such as mycotoxins, metals, and other air pollutants). It is, therefore, imperative that an effort be made not only to identify and possibly remove an increasing number of carcinogens from the environment, but also, and concurrently, to identify steps in their mode of penetration into the organisms and the target tissues and in their critical interaction with cell constituents so that these steps could be exploited as target points for inhibitory or protective measures. The considerable development of refined technical methods presently available for the identification of these critical steps in carcinogenesis by studies in analytical chemistry, biochemistry, cell biology, molecular biology, and immunology can now be brought to bear on our ability to develop useful, protective measures against the ultimate effects of the carcinogenic process. The identification of critical parameters required for the carcinogenic action of certain groups of chemicals, obtained in animal studies, also represents the only direct link that can be applied to the study of the susceptibility of man to comparable exposure conditions.

For example, the establishment of a strong correlation between the enzymatic activation of certain chemicals to the proximate carcinogen by means of given groups of enzymes, such as the microsomal aryl hydroxylase for polynuclear hydrocarbons may lead to the identification of groups of individuals as being highly susceptible to certain specific carcinogenic exposures, pharmacologic inhibition of some of these enzyme systems may lead to a marked decrease of the cancer risk for such individuals. As other examples, analogous approaches can be made at the level of the control of protein synthesis in differentiation-dependent carcinogenic mechanisms (e.g. hormone-dependent tumors and vitamin A-dependent tumors) and at the level of the immunological control of the growth of transformed cells. A broad front of attack can thus be developed against the sequence of events required for the carcinogenic process to lead to the establishment of progressively growing invasive tumors.

The effective development of such studies depends in part on the availability of appropriate animal models, capable of reproducing tumor types analogous to those observed in man. The development of appropriate biological models is particularly important for the establishment of selectively sensitive bioassay systems. An important phase of the program consists in the selection and standardization of bioassay systems that could be reproduced in several laboratories under strictly controlled standardized conditions. This is an essential requirement if we want to collect and analyze large series of data on carcinogenesis tests. For this purpose, a detailed plan is being worked out for a data retrieval and analysis system for carcinogenesis studies. The present overall Program Plan in Chemical Carcinogenesis and Prevention of Cancers constitutes the basis for the development of detailed operational plans aimed at the implementation of each of its phases.

2. *Special Lung Cancer Program*

In the course of the current year, the Associate Scientific Director for Carcinogenesis has continued to participate in the activity of the Tobacco Working Group of the Lung Cancer Task Force and to chair its Subcommittee on Bioassays. The Tobacco Working Group, under the chairmanship of the Director, NCI, has made recommendations aimed at the development of a less hazardous cigarette. A plan has been established in FY 1969 following these recommendations. This activity is articulated in the following phases: selection, preparation, and characterization of different types of tobacco and tobacco smoke condensates for subsequent bioassay and analysis; bioassay of tobacco smoke and its fractions in a battery of animal systems; chemical analysis of the tobacco smoke and its fractions; evaluation of the data obtained in relation to the human situation.

An appropriation was made to the Lung Cancer Task Force for use in the Fiscal Year 1969. From it, $1,153,000 was assigned to the Carcinogenesis Area, Etiology, to implement the initial phase of this program. Following the recommendation of the Tobacco Working Group, a balanced program has been developed to include preparation and analysis of different types of tobacco smoke condensates; bioassays for carcinogenicity by mouse skin application (by ingestion and by direct administration into the respiratory tract); and bioassays for cytotoxic and ciliary-inhibitory effects on *in vitro* and *in vivo* systems. An additional research program, later to be funded directly through the Carcinogenesis Program was developed to characterize, define and standardize the conditions of exposure to respiratory carcinogens in the presence of vehicles and particles of different physico-chemical characteristics. A study was set up to obtain a definition of the kinetics of the cellular response to the respiratory administration of particulates and carcinogens. In addition, a study was developed to characterize and define the observed inhibition of squamous metaplasia and squamous carcinoma development in the respiratory tract under the effect of high levels of vitamin A. Collaborative programs are also being used to develop additional studies related to the definition of bioassay models for respiratory carcinogenesis and co-carcinogenesis. The Lung Cancer Unit, OASDC, has been established as the focus of intramural support to this program.

3. *Outline of Five-Year Plan for Carcinogenesis*

The Carcinogenesis Program will be responsible for planning, implementing, and managing the coordinated research and testing program on cancer induction by chemical and physical factors and on its prevention in man. In order to meet this responsibility, three main program approaches have been established for (a) identification of carcinogenic activity of selected agents by bioassays; (b) development and selection of biological models for bioassays and for the characterization of carcinogenesis processes, with emphasis on organ-specific cancer models; (c) identification of processes required for the carcinogenic action and for development of cancers, as target points for corrective measures.

The Program will be managed through five principal segments of operation. The five segments represent the major operative components of the convergence plan developed for the Carcinogenesis Area at NCI and they will be closely correlated with each other. The five segments are as follows: (1) Bioassays, (2) Biological Models, (3) Chemistry and Molecular Carcinogenesis, (4) Biology and Immunology, and (5) Resources and Information.

Segment 1 - Bioassays: This Segment will provide selection and identification of carcinogens which may be responsible for the induction of cancer in man. A series of bioassay systems will include several species and strains of animals and major routes of administration, i.e. ingestion, skin application, respiratory administration, etc. Each of the test systems will be standardized and monitored for internal consistency. Through each of the bioassay systems, the program will conduct long-term animal carcinogenesis bioassays for the identification of carcinogenic activity of chemical and physical agents. The agents will be selected with the collaboration of the Resources and Information Segment; they will be subjected to acute toxicity studies and then entered into long-term carcinogenesis bioassays. The main categories of chemicals to be bioassayed will include: (1) tobacco smoke types (to identify a less hazardous cigarette); (2) pesticides and other agricultural chemicals; (3) food additives and contaminants; (4) occupational hazards; (5) industrial pollutants and industrial wastes; (6) household products; and (7) natural products.

The bioassays will be continually monitored and tumor-induction characteristics will be identified and correlated to the evaluation of the biological model systems affected, and to the potential risks for the human population. This represents a major national resource for the identification of carcinogenic agents among environmental chemicals. Its thrust is not only that of recognizing potential hazards but also that of relating them to specific cancer patterns in the animals and in man. This acquisition of results of a large-scale bioassay program on thousands of chemicals will constitute also a necessary baseline of data against which to test the validity of short-term bioassay models that will be developed and of preventive or inhibitory measures developed through other phases of the program. Major resources will be developed though commercial contractors, and with the establishment of large testing centers.

Segment 2 - Biological Models: The operation of this Segment will be aimed at the identification, development and selection of biological models to be used for carcinogenesis bioassays and for studies on the characterization of the carcinogenic processes and their prevention. The animal models will provide the closest possible counterpart to the main human cancer types, thus allowing selective identification of causative agents and conditioning host factors, required for the development of the main cancer types. The Segment will (a) conduct studies to establish feasibility of developing organ-specific animal cancer models; (b) establish quantitative and qualitative ranges of applicability and develop appropriate reference standards; (c) pretest the model systems to determine their reproducibility and ranges of responsiveness to groups of known carcinogens and non-carcinogenic analogs; (d) define the main host factors responsible for the biological characterization of the response of specific tissues to carcinogens; and (e) develop methods for comparison of both cellular and tissue responses in specific organ systems in animals and man. The main programs in this Segment will be developed in close collaboration with the organ-specific Task Forces. A sizable operation is already underway for the Lung Cancer Program.

A program is being developed for cancer of the colon and other parts of the gastro-intestinal tract. Additional programs will be developed for endocrine-related cancer (breast, ovary, prostate, etc.) childhood cancers, cancer of the bladder and kidney, cancer of the pancreas, and others.

The main resources recruited for collaboration to these programs will be in the areas of animal and human pathology, cell biology, endocrinology, embryology, and experimental pharmacology and toxicology.

Segment 3 - Chemistry and Molecular Carcinogenesis: The program of this Segment is aimed at an understanding and definition of the key biochemical processes in cell growth control and in the interaction of carcinogens with cell constituents, that are potentially exploitable targets for the inhibition of carcinogenesis.

The major efforts will be devoted to expand studies in the following areas: (1) Enzymatic control mechanisms of the activation and metabolism of chemical carcinogens (particular emphasis will be given to defining the role of microsomal enzymes in different cell types for different carcinogens). (2) Vertebrate somatic cell genetics (induction, selection, and propagation of somatic cells which are defective in specific biochemical pathways). (3) The role of nucleic acids metabolism and particularly the role of DNA repair mechanisms in maintaining functional integrity of the cell following DNA damage induced by radiation and chemical carcinogens. (4) The role of cell membranes in the penetration and interaction of carcinogens in their target cells.

Segment 4 - Biology and Immunology: The goal of the Segment will be to develop and apply immunological tools for the detection of the effects of carcinogens, the control of cancer induction, and the study of *in vitro* carcinogenesis. Known immunological methods and approaches should be applied to a wide variety of carcinogenesis systems to detect environmental factors that cause or augment cancer induction. Cancer antigens induced by carcinogens will be isolated and characterized to obtain reagents for these studies. Current knowledge permits the extension of such studies directly to man. The Segment will also explore the value of immunotherapy methods (specific and nonspecific) to the control of the induction and development of certain forms of cancer. Animal models for immunological control of cancer will be developed, with emphasis on identification of cell antigens and of specific immune defenses (both humoral and cellular) on the evaluation of immune reaction potential and on their applicability to the monitoring of human populations.

The *in vitro* interaction of carcinogens with individual cells provides a major tool in investigating the biology and chemistry of the process of transformation from normal to cancer cells. Knowledge derived from animal cell models should be applied to studies of *in vitro* carcinogenesis of human cells. *In vitro* studies of co-carcinogenesis, particularly of the interactions of chemical and viral carcinogens will be conducted. *In vitro* models of carcinogenesis will be developed to screen potential environmental carcinogens and to study the interaction between chemical carcinogens and factors that regulate cell growth and differentiation, and to study early events in carcinogenesis, especially in macromolecular synthesis and ultrastructural changes.

Segment 5 - Resources and Information: This Segment will function as a resource for the entire program. Its main components will be the following: (1) Chemical Resources: (a) centralized service for the preparation and distribution of highly purified and standardized samples of carcinogens and related compounds; (b) analytical chemical resources for the development and utilization of methods required for the identification of carcinogens in biological tissues and in environmental samples; (c) chemical preparative resources required to prepare specialized materials for testing in the in the Bioassay Segment. (2) Animal resources that will provide standardized animal colonies of selected species and strains, monitored and controlled for health conditions and reproducibility. (3) Registry of Comparative Tumor Pathology, which will provide (a) coordination for diagnostic tumor pathology and standardized classifications necessary for the evaluation of pathology results in bioassays and other studies; (b) documentation of pathology standards; and (c) a comparative pathology reference center for the close coordination of pathology criteria used throughout the program. (4) Another major portion of this Segment will be devoted to the development of information resources. These will include (a) the retrieval and compilation of summaries of literature data in chemical carcinogenesis; (b) the continuation of *Survey of Compounds That Have Been Tested for Carcinogenic Activity;* and (c) the development of computerized systems and the necessary input formats for the retrieval and analysis of bioassay data originated by the program. (5) Another major complement of the Segment will be the further development of a selection and ranking system for the purpose of establishing priorities for testing of environmental chemicals selected out of the hundreds of thousands that enter into contact with man. (6) The Information System will also include a continually up-dated list of ongoing carcinogenesis bioassays to be maintained on an international basis as a means for avoiding duplication of testing.

C. *MANAGEMENT ACTIVITIES*

1. *Monitoring and Reviewing*

The collaborative work supported by contracts in the Carcinogenesis Program and in the Lung Cancer Task Force are coordinated through the Office of the Associate Scientific Director for Carcinogenesis. The Program and Data Analysis Unit, OASDC, provides support for the overall contract management program from the point of view of scientific information, documentation of ongoing levels of effort in each program area, and systematic data analysis. Project officers, selected from the staff of the Carcinogenesis Area, are assigned to each contract. The project officer provides scientific guidance, program relevance and overall direction to the project, and monitors its performance, reporting directly to the Associate Scientific Director for Carcinogenesis. Most valuable collaboration was provided by the Associate Scientific Director for Program, Etiology, who served as project officer on several important contracts.

A new position has been established for a Coordinator for Collaborative Research. Dr. Allen H. Heim has assumed this position in February 1971. With his collaboration the Area is now working out the detailed plan for a more effective contract management operation, by establishing Program Segments that will acquire overall planning and monitoring functions within each sub-area of the Program. This system has proven very effective in the development of the Special Virus Cancer Program of Etiology.

At present, contract proposals are first reviewed by staff and by ad hoc committees appointed by the Scientific Director for Etiology including members from other parts of NCI or NIH. They are also reviewed for relevance, need, and priority; this review was done until recently by the Etiology Program Management Group. Now this function has been transferred to the newly appointed Interim Collaborative Program Review Group. The selected proposals and budgets are submitted for technical review and recommendation to the Chemical Carcinogenesis Contract Review Committee. Final recommendation on priority and funding is made by the Associate Scientific Director for Carcinogenesis to the Scientific Director for Etiology.

The contract specialists provide coordination between scientific management and administrative mechanisms of negotiation, award and fiscal monitoring of contracts. They provide an essential and most effective function for the implementation of the complex research programs in the Area.

2. *Fiscal Summary*

The following charts and tables report the present distribution of funds by activities in the Carcinogenesis Area, including Direct Operations and Contracts. It can be added that the total amount of NCI grant funds allocated to carcinogenesis projects (non-viral), was $3,729,000 for FY 1970.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES IN THE CARCINOGENESIS PROGRAM

TYPE OF ACTIVITY		ANNUAL LEVEL[1/]		(PERCENT)
TOTAL		$7,341,632		(100.0)
I. Carcinogenesis Bioassays		3,468,199		47.2%
A. Food Constituents and Contaminants	545,830		15.7%	
B. Air Pollutants	159,000		4.6%	
C. Tobacco and Smoking Products	1,087,932[2/]		31.4%	
D. Pharmacologic Agents	311,058		9.0%	
E. Industrial Occupational Hazards	279,121		8.0%	
F. Pesticides	506,730		14.6%	
G. Other Environmental Agents	578,528		16.7%	
II. Biological Models for Carcinogenesis		2,224,039		30.3%
A. Lung Cancer	1,344,372		60.4%	
B. Other Models	879,667		39.6%	
III. Processes of Carcinogenesis		745,094		10.2%
IV. Resources		347,555		4.7%
V. Information		556,745		7.6%

[1/] Funds obligated during twelve-month period ending April 15, 1971. Not included in this amount are ongoing activities for which no new funds are obligated. See Table II for individual contracts involved.

[2/] Including $896,000 from Lung Cancer Task Force Appropriation.

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY IN THE CARCINOGENESIS PROGRAM

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]	TOTAL[1/]
I. Carcinogenesis Bioassays		$3,468,199
A. Food Constituents and Contaminants		545,830
Bionetics Research Labs.(NIH-69-2085)	17,740	
Bio-Research Consultants(PH43-68-1311)	6,750	
California, University of(NIH-70-2206)	104,720	
City of Hope Medical Center(PH43-65-1036)	15,680	
Columbia University(PH43-67-731)	57,555	
General Foods Corporation(PH43-68-1005)	25,788	
Oregon State University(PH43-68-1462)	5,597	
Nebraska, University of(PH43-68-959)	312,000	
B. Air Pollutants		159,000
Battelle-Northwest(PH43-68-1372)	75,000	
Nebraska, University of(PH43-68-959)	84,000	
C. Tobacco and Smoking Products		1,087,932
American Health Foundation(NIH-70-2087)	258,175*	
Battelle-Northwest(PH43-68-1372)	76,477	
Hazleton Laboratories(NIH-69-2145)	89,570*	
Hazleton Laboratories(NIH-69-2149)	256,250*	
Little, Arthur D, Inc.(NIH-69-2147)	67,900*	
Mel-Labs, Inc.(NIH-69-2084)	263,725*	
New York University(PH43-64-938)	75,835	
D. Pharmacologic Agents		311,058
Bionetics Research Labs.(NIH-69-2085)	62,104	
Nebraska, University of(PH43-68-959)	24,000	
Southern Research Institute(PH43-68-998)	224,954	
E. Industrial Occupational Hazards		279,121
Bionetics Research Labs.(NIH-69-2085)	8,870	
Bio-Research Consultants(PH43-68-1311)	162,308	
Cincinnati, University of(PH43-64-45)	6,300	
Nebraska, University of(PH43-68-959)	24,000	
New York University(NIH-71-2020)	46,000	
San Francisco, University of(PH43-64-886)	31,643	
F. Pesticides		506,730
Bionetics Research Labs.(NIH-69-2085)	53,230	

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]	TOTAL[1/]
Cincinnati, University of(PH43-64-45)	6,300	
Gulf South Research Institute (NIH-70-2210)	212,600	
Hazleton Laboratories(NIH-70-2209)	222,600	
Nebraska, University of(PH43-68-959)	12,000	
G. Other Environmental Agents		578,528
Bionetics Reserach Labs.(NIH-69-2085)	8,870	
Cincinnati, University of(PH43-64-45)	56,400	
Mass. Institute of Technology (PH43-62-468)	140,600	
Nebraska, University of(PN43-68-959)	144,000	
New York University(NIH-71-2020)	45,481	
San Francisco, University of (PH43-64-886)	31,000	
Temple University(PH43-65-1029)	87,177	
Tennesse, University of(NIH-69-2077)	65,000	
II. Biological Models for Carcinogenesis		$2,224,039
A. Lung Cancer		1,344,372
Atomic Energy Comm.(FS-13)	400,000	
Atomic Energy Comm.(40-117-67)	0	
California, University of(NIH-70-2218)	96,065	
IIT Research Institue(NIH-69-2148)	148,705	
Marquette School of Medicine (NIH-69-2082)	67,000	
Mass. Institute of Technology (NIH-69-2083)	81,400	
Nebraska, University of(PH43-68-959)	200,000	
New York University(PH43-66-962	278,221	
Ohio State University(NIH-69-2144)	72,981	
B. Other Models		879,667
Chicago, University of(NIH-69-2087)	245,100	
Mel-Labs., Inc.(NIH-70-2201)	130,600	
Microbiological Association (NIH-70-2199)	246,262	
Nebraska, University of(NIH-70-2203)	96,425	
Nebraska, University of(PH43-68-959)	161,280	

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

ACTIVITY CONTRACT	DOLLAR LEVEL[1]	TOTAL[1]
III. Processes of Carcinogenesis		$ 745,094
Atomic Energy Comm. (FS-13)	200,000	
Cordis Corporation (NIH-69-2076)	90,674	
Life Sciences (NIH-69-2056)	142,100	
Nebraska, University of (PH43-68-959)	240,320	
Temple University (PH43-64-504)	21,000	
Weizmann Institute (NIH-70-2217)	51,000	
IV. Resources		$ 347,555
Ash Stevens (PH43-66-929)	126,170	
Battelle-Columbus (NIH-69-2251)	18,075	
Charles River Breeding Labs. (PH43-66-87)	11,510	
IIT Research Institute (NIH-70-2245)	71,203	
Mass. Institute of Technology (NIH-70-2180)	45,700	
Minnesota, University of (NIH-70-2058)	5,000	
Southwest Research Institute (PH43-65-1000)	69,897	
V. Information		$ 556,745
Franklin Institute (NIH-71-2073)	44,200	
Sci. Literature Corp. (NIH-70-2036)	46,740	
Stanford Research Institute (NIH-71-2045)	309,455	
Thompson, John I, Co. (NIH-69-2086)	156,350	
GRAND TOTAL		$7,341,632

[1] Funds obligated during twelve-month period ending April 15, 1971. Not included in this amount are ongoing activities for which no new funds were obligated.

*Supported from the Lung Cancer Task Force Appropriation.

TABLE III

ALPHABETICAL LIST OF CONTRACTS AND ACTIVITY DESCRIPTION IN THE CARCINOGENESIS PROGRAM

CONTRACT ACTIVITY		DOLLAR LEVEL[1]	TOTAL[1]
American Health Foundation (NIH-70-2087)			$ 258,175
IC	Bioassay--Tobacco & Smoking Products	258,175	
Ash Stevens (PH43-66-929)			126,170
IV	Resources	126,170	
Atomic Energy Commission (FS-13)			600,000
III	Processes of Carcinogenesis	200,000	
IIA	Biological Models--Lung Cancer	400,000	
Battelle-Northwest (PH43-68-1372)			151,477
IB	Bioassays--Air Pollutants	75,000	
IC	Bioassays--Tobacco & Smoking Products	76,477	
Battelle-Columbus (NIH-69-2251)			18,075
IV	Resources	18,075	
Bionetics Reserach Lab. (NIH-69-2085)			150,814
IA	Bioassays--Food	17,740	
ID	Bioassays--Pharmacologic Agents	62,104	
IE	Bioassays--Industrial Occupational	8,870	
IF	Bioassays--Pesticides	53,230	
IG	Bioassays--Other Environmental Agents	8,870	
Bio-Research Consultants (PH43-68-1311)			169,058
IA	Bioassays--Food	6,750	
IE	Bioassays--Industrial Occupational	162,308	
California, University of (NIH-70-2206)			104,720
IA	Bioassays--Food	104,720	
California, University of (NIH-70-2218)			96,065
IIA	Biological Models--Lung Cancer	96,065	
Charles River Breeding Labs. (PH43-66-87)			11,510
IV	Resources	11,510	
Chicago, University of (NIH-69-2087)			245,100
IIB	Biological Models--Other Models	245,100	

TABLE III (CONTINUED)

CARCINOGENESIS PROGRAM

CONTRACT ACTIVITY		DOLLAR LEVEL[1]	TOTAL[1]
Cincinnati, University of (PH43-64-45)			69,000
IE	Bioassay--Industrial Occupational	6,300	
IF	Bioassays--Pesticides	6,300	
IG	Bioassays--Other Environmental Agents	56,400	
City of Hope Medical Center (PH43-65-1036)			15,680
IA	Bioassays--Food	15,680	
Columbia University (PH43-67-731)			57,555
IA	Bioassays--Food	57,555	
Cordis Corporation (NIH-69-2076)			90,674
III	Processes of Carcinogenesis	90,674	
Franklin Institute (NIH-71-2073)			44,200
V	Information	44,200	
General Foods Corporation (PH43-68-1005)			25,788
IA	Bioassays--Food	25,788	
Gulf South Research Institute (NIH-70-2210)			212,600
IF	Bioassay--Pesticides	212,600	
Hazleton Labs. (NIH-69-2145)			89,570
IC	Bioassay--Tobacco & Smoking Products	89,570	
Hazleton Labs. (NIH-69-2149)			256,250
IC	Bioassays--Tobacco & Smoking Products	256,250	
Hazleton Labs. (NIH-70-2209)			222,600
IF	Pesticides	222,600	
IIT Research Institute (NIH-70-2245)			71,203
IV	Resources	71,203	
IIT Research Institute (NIH-69-2148)			148,705
IIA	Biological Models--Lung Cancer	148,705	
Life Sciences (NIH-69-2056)			142,100
III	Processes of Carcinogenesis	142,100	

TABLE III (CONTINUED)

CARCINOGENESIS PROGRAM

CONTRACT ACTIVITY		DOLLAR LEVEL[1/]	TOTAL[1/]
Little, Arthur D, Inc. (NIH-69-2147)			67,900
IC	Bioassay--Tobacco & Smoking Products	67,900	
Marquette School of Medicine (NIH-69-2082)			67,000
IIA	Biological Models--Lung Cancer	67,000	
Mass. Institute of Technology (PH43-62-468)			140,600
IG	Bioassay--Other Environmental Agents	140,600	
Mass. Institute of Technology (NIH-69-2083)			81,400
IIA	Biological Models--Lung Cancer	81,400	
Mass. Institute of Technology (NIH-70-2180)			45,700
IV	Resources	45,700	
Mel-Labs, Inc. (NIH-69-2084)			263,725
IC	Bioassay--Tobacco & Smoking Products	263,725	
Mel-Labs, Inc. (NIH-70-2201)			130,600
IIB	Biological Models--Other Models	130,600	
Microbiological Associates (NIH-70-2199)			246,262
IIB	Biological Models--Other Models	246,262	
Minnesota, University of (NIH-70-2058)			5,000
IV	Resources	5,000	
Nebraska, University (PH43-68-959)			1,201,600
IA	Bioassays--Food	312,000	
IB	Bioassays--Air Pollutants	84,000	
ID	Bioassays--Pharmacologic Agents	24,000	
IE	Bioassays--Industrial Occupational	24,000	
IF	Bioassays--Pesticides	12,000	
IG	Bioassays--Other Environmental Agents	144,000	
IIA	Biological Models--Lung Cancer	200,000	
IIB	Biological Models--Other Models	161,280	
III	Processes of Carcinogenesis	240,320	
Nebraska, University of (NIH-70-2203)			96,425
IIB	Biological Models--Other Models	96,425	

TABLE III (CONTINUED)

CARCINOGENESIS PROGRAM

CONTRACT	ACTIVITY	DOLLAR LEVEL[1]	TOTAL[1]
New York University (PH43-64-938)			75,835
	IC Bioassays--Tobacco & Smoking Products	75,835	
New York University (PH43-66-962)			278,221
	IIA Biological Models--Lung Cancer	278,221	
New York University (NIH-71-2020)			91,481
	IE Bioassays--Industrial Occupational	46,000	
	IG Bioassays--Other Environmental Agents	45,481	
Ohio State University (NIH-69-2144)			72,981
	IIA Biological Models--Lung Cancer	72,981	
Oregon State University (PH43-68-1462)			5,597
	IA Bioassay--Food	5,597	
San Francisco, University of (PH43-64-886)			62,643
	IE Bioassay--Industrial Occupational	31,643	
	IG Bioassay--Other Environmental Agents	31,000	
Sci. Literature Corp. (NIH-70-2036)			46,740
	V Information	46,740	
Southern Research Institute (PH43-68-998)			224,954
	ID Bioassay--Pharmacologic Agents	224,954	
Southwest Research Institute (PH43-65-1000)			69,897
	IV Resources	69,897	
Stanford Research Institute (NIH-71-2045)			309,455
	V Information	309,455	
Temple University (PH43-65-1029)			87,177
	IG Bioassay--Other Environmental Agents	87,177	
Temple University (PH43-64-504)			21,000
	III Processing of Carcinogenesis	21,000	
Tennesse, University of (NIH-69-2077)			65,000
	IG Bioassay--Other Environmental Agents	65,000	

TABLE III (CONTINUED)

CARCINOGENESIS PROGRAM

CONTRACT ACTIVITY		DOLLAR LEVEL[1]	TOTAL[1]
Thompson, John I., Co. (NIH-69-2086)			156,350
V	Information	156,350	
Weizmann Institute (NIH-70-2217)			51,000
III	Processing of Carcinogenesis	51,000	
	GRAND TOTAL	$7,341,632	$7,341,632

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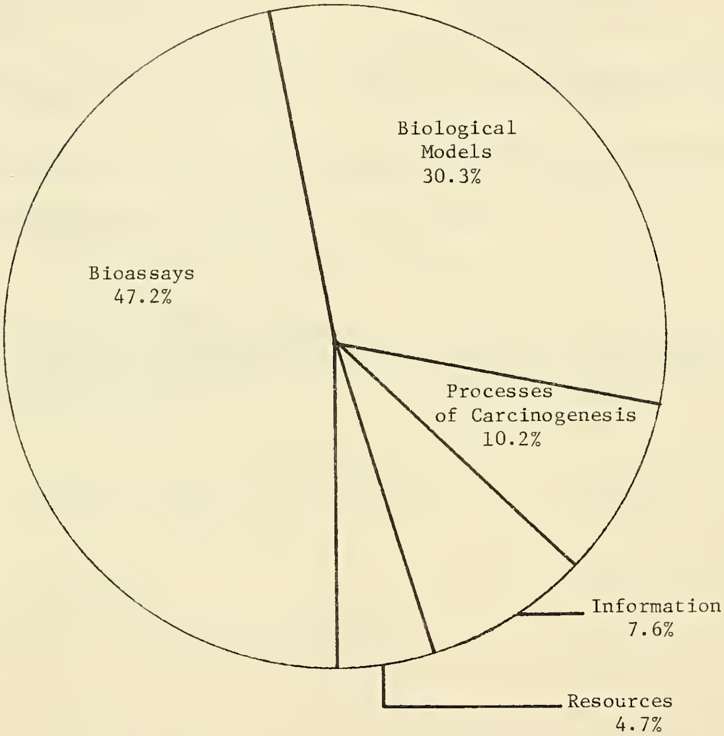
[1] Funds obligated during twelve-month period ending April 15, 1971. Not included in this amount are ongoing activities for which no new funds were obligated. See Table II for individual contracts involved.

CARCINOGENESIS
ETIOLOGY AREA, NCI

DISTRIBUTION OF CONTRACT FUNDS[1]
FISCAL YEAR 1971



Total Contract Funds: $7,341,632

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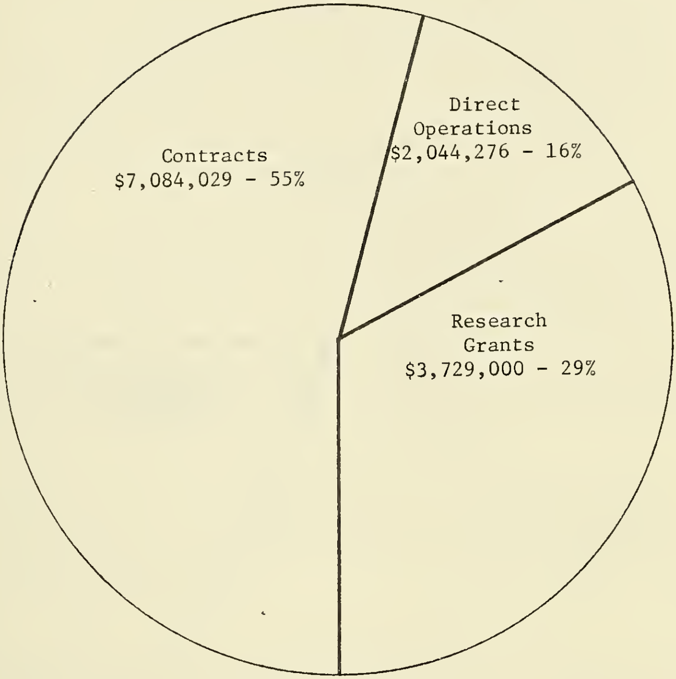
[1] Funds obligated during twelve-month period ending April 15, 1971.

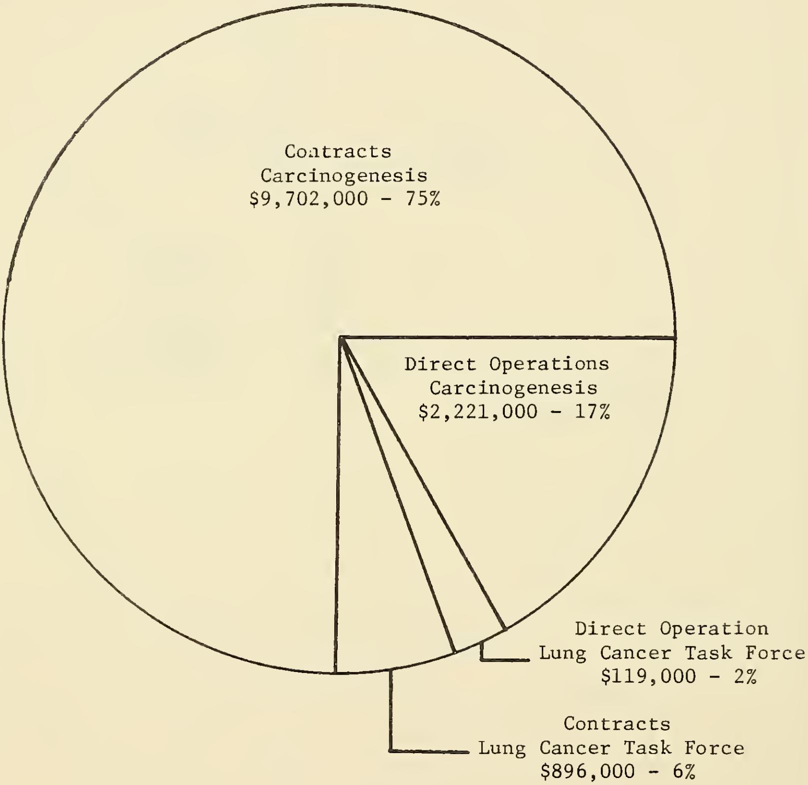
MAJOR SUPPORT MECHANISMS FOR CARCINOGENESIS PROGRAMS IN THE NATIONAL CANCER INSTITUTE FISCAL YEAR 1970



Total funding for Carcinogenesis programs, NCI, FY 1970: $12,857,305

CARCINOGENESIS
ETIOLOGY AREA, NCI

FUNDING OBLIGATIONS[1] - DIRECT OPERATION AND CONTRACT
FISCAL YEAR 1971



Total Contract:	$10,598,000
Total Direct Operation:	2,340,000
TOTAL FUNDING:	$12,938,000

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[1] Projected through the end of Fiscal Year 1971

D. *SCIENTIFIC ACTIVITIES: SUMMARY REPORT AND PROGRESS HIGHLIGHTS*

The progress accomplished in the Carcinogenesis Area in Fiscal Year 1971 covers a broad front of attack to the problem of the identification of causative factors and the prevention of cancers. Many of the projects in this Area have been underway for several years and represent a patient, intensive, and costly long-term effort towards the attainment of our specific program goals. Work in chemical carcinogenesis is characterized by the two-three year duration of each lifespan study in common laboratory rodents, with the resulting long range commitment of efforts and resources. Other projects represent a complex effort to develop and refine the research methods necessary for studying the spectrum of chemical, biochemical, biological, and molecular mechanisms that are responsible for the host response to chemical and physical carcinogens, and for the development of preventive measures.

Summary reports of the Office of the Associate Scientific Director and of the Branches, as well as detailed reports of individual projects and contracts will be presented in the following sections of this Annual Report. The highlights of these reports are summarized here in the perspective of the overall plan of operation of the Chemical Carcinogenesis Area. The Program Plan in Chemical Carcinogenesis, developed in FY 1969, was the basis for the establishment of new projects. However, some long-term projects that were underway before the Plan was implemented, need to be brought to an orderly conclusion; therefore, some of the ongoing projects and contracts can only indirectly be related to the Plan's design.

The following summary of progress highlights was prepared prior to testimony by the Director, NCI, at the 1971 Congressional Budget Hearings. The highlights are included in this report for the convenience of the reader as concise statements of some important areas of progress within the Carcinogenesis Program during the past year.

1. *Program Planning*

(a) We have instituted the position of Coordinator for Collaborative Research in the Office of the Associate Scientific Director for Carcinogenesis to make it possible to develop a detailed plan for establishing Program Segments for maximum competence and efficiency in the development of each major portion of the program. Each segment will have a team of science managers working together with leading staff scientists and with an advisory group to develop, monitor, and review all the projects in that segment. Implementation of this plan is imminent.

Importance: The number of contracts in Carcinogenesis has increased from less than 30 to near 80 in the last three years. A more articulated management structure is essential for maximum efficiency in implementation and for future developments. Increased advisory inputs from the scientific community will be possible at the segment level.

Future Plans: To set up a management staff and an advisory review group in support of each major program segment.

(b) Special Cancer Programs have been planned. The Special Lung Cancer Program, now in its second year, has followed the previously planned directions: it includes a tobacco testing and research program (which now absorbs all the funds appropriated under the Lung Cancer Task Force) as well as a program on the development and definition of biological models for respiratory carcinogenesis and on the identification and inhibition of its key processes (which is now totally funded from the Carcinogenesis Program).

A Colon Cancer Etiology Program is in an advanced stage of formal planning with the support of an ad hoc Colon Carcinogenesis Review Group. Contracts have been already developed according to the outline of this plan.

Preliminary planning discussions have been made towards programs of bladder cancer, childhood cancers, endocrine-related cancers and cancer of the pancreas.

Importance: The above listed major types of cancers account for a large proportion of all deaths by cancer in the United States. A well-coordinated, targeted attack on each of the major types of cancers is of the utmost importance in the framework of a national effort towards the conquest of cancer. Biological model systems, reproducing the main characteristics of these forms of cancer as seen in man, are essential for a selective attack on the identification and inhibition of the causative agents and specific host responses. They will provide a much needed basis for comparing the animal response to that observed in man at the morphologic, biochemical, and immunological levels. Ultimately, our ability to predict levels of carcinogenic risk in man and to identify susceptible and resistant individuals will depend on such specialized comparisons of response in animals and man.

Future Plans: To develop plans for special multidisciplinary organ-oriented programs for each of the major categories of cancer seen in man, through collaborative research backed up by competent core groups in the staff. Review conferences of leading experts, such as the two we sponsored for the Lung Cancer Program in FY 1970, will be considered for the other special programs.

2. *Information Systems*

(a) Systems are developed to coordinate carcinogenesis information from various sources and to serve as a basis for selection and design of future studies. Specific ongoing projects include (1) collection, publication, and dissemination of information in the literature on carcinogenesis studies (*Carcinogenesis Abstracts*, a monthly publication [Contractors: Scientific Literature, and Franklin Institute]) and on carcinogenesis bioassays (*Survey of Compounds That Have Been Tested for Carcinogenic Activity* [Contractor: J. I. Thompson & Co.]); (2) development of a comprehensive automated (computer) data system for all bioassay studies, now undergoing field trials; and (3) data processing and analysis support for special studies.

Importance: The systems will provide us with almost immediate access to information essential to the functions of monitoring current studies, evaluating results and planning future studies, in carcinogenesis. The system will also enable us to identify those chemicals which have been tested, and the adequacy of such tests (as reported in the literature), a necessary basis for the development of a priority listing of those chemicals which still need to be tested in future studies.

Future Plans: To continue these efforts and to further develop the bioassay data system.

(b) A new project has been implemented in the current Fiscal Year (Contractor: Stanford Research Institute) to define the feasibility of a systematic methodology for information retrieval leading to ranking of environmental chemicals for carcinogenesis testing. This selection will be based on the estimated risk for potential chemical carcinogenic hazards to man. The first series of studies have been directed toward the examination of the system structure, the main categories of exogenous potential sources of carcinogens, and the availability of production and usage data.

Importance: This is the first systematic attempt to generate a priority ranking for the selection of a few hundred compounds to be entered on test out of many thousands of potential candidates. The project will also generate a compendium of the best available knowledge of potential risks from yet untested compounds.

Future Plans: To complete the feasibility study and to activate such a program on a continuing basis.

(c) Carcinogenesis staff participates in the international project sponsored by the International Agency for Research in Cancer (IARC), WHO, Lyon, France, to produce monographs on individual chemical carcinogens with a documentation of their biological activity and risk for man. The monographs are prepared by recognized authorities and reviewed by an international expert committee. Close collaboration has been established between OASDC staff and the IARC staff on this project.

Importance: This effort will provide a documentation, reviewed by qualified international experts, on the evaluation of risks from selected chemical carcinogens.

Future Plans: To develop a continuing collaboration with IARC and to provide documentation for use of the international expert committee.

3. *Resources*

(a) Preparative and synthetic chemical resources have been developed to meet specific program needs. They include (1) preparation of particulate carriers for respiratory carcinogenesis studies in specified size ranges having specific physical characteristics (Contractor: IIT Research Institute); (2) preparation of cigarette smoke condensates from many varieties of tobacco cigarettes and from marijuana cigarettes, under standardized conditions for use in the bioassay program (Contractor: Mel. Labs.); and (3) synthesis of nucleotides and oligonucleotides which will irreversibly inactivate the enzyme which destrous newly synthesized RNA in the nucleus (Contractor: Ash Stevens, Inc.)

Importance: Resources capable of providing the Program with standardized preparations for use in bioassays or with high quality capability for the synthesis and production of needed reagents are indispensible in the operation of a complex targeted collaborative program.

Future Plans: To continue present activities and to establish a new resource providing purified reference samples of carcinogens and analogs for use by many contractors: this resource is being set up with the collaboration of CCNSC.

(b) Analytical chemical resources have been established to provide information on products or compounds of importance as environmental carcinogens. One project is concerned with developing methods for the detection of N-nitroso compounds in foodstuffs: methods have been devised to isolate relatively volatile nitrosamines from food samples (Contractor: Massachusetts Institute of Technology). Another is using the method for detection of N-nitrosamines in fresh tobacco smoke, developed in the past year, to analyze smoke from different types of cigarettes: the results indicate that the potent carcinogen, dimethylnitrosamine, is present in tobacco smoke and that its level varies with the type of tobacco (Contractor: Southwest Research Institute). A project for the development of analytical methods for the chemical characterization of tobacco smoke is actively continuing (Contractor: (AEC, Oak Ridge National Laboratory). Other analytical studies are direct components of a variety of projects in several segments of the program.

Importance: Development of a method for the identification of nitrosamines in food and in tobacco smoke represents a major advance in the evaluation of the environmental hazards of this class of powerful carcinogens. The analytical characterization of tobacco and other products that are submitted to bioassay is required for the proper evaluation of the factor(s) responsible for biological activity in the tested products.

Future Plans: To continue and increase the efforts aimed at establishing an "environmental profile" of the distribution of known or suspected carcinogens and at defining the chemical composition of tested materials.

4. *Carcinogenesis Bioassays*

Bioassay screening of several groups of environmental chemicals has been conducted through research contracts. Approximately 47% of the Carcinogenesis contract funding (including Task Force appropriations) is now used for direct bioassay operations. At this level of funding, approximately 250 compounds are presently under test. It is recognized that information on the carcinogenicity of chemicals in animal systems will not provide direct extrapolation to the hazards in man. Moreover, it is predictable that a large number of agents, even though identified as carcinogenic, will be difficult or impossible to remove, in the foreseeable future, from the environment of man. Despite these restrictions, the results obtained from critical animal studies constitute at present the only direct evidence that indicate potential hazards from environmental chemical carcinogens. In addition, data on the carcinogenic activity of a large variety of chemicals in different species and tissues are needed in order to define and validate the specificity of any new biological system developed for a more selective study of carcinogenic effects, such as *in vitro* systems. Finally, large scale standardized bioassays will provide a basis for detecting patterns of carcinogenic response in large animal populations, obtained by pooling observations on many individual tests under identical experimental conditions.

Negotiation of several large new contracts for carcinogenesis bioassays is under way. These contracts will be initiated prior to the end of this Fiscal Year.

It is essential to stress that a considerable lag time elapses between the first report of a suspiciously high incidence of tumors in the course of a bioassay, and the time when all the necessary critical information, required for a conclusive statement of carcinogenicity of the test compound, is collected and reviewed. This includes completion of the experimental protocol for the treated animals and for their controls, (which usually live longer), completion of the histopathological examination of all relevant material and analysis of all the results with proper biometric methods. Publication of conclusive reports therefore follows with considerable delay the first reports of positive findings: until the conclusive reports are completed, the information on newly detected carcinogenic effects must be considered "soft" and therefore "informal". With this essential proviso, the main findings obtained this year in the bioassay operations are listed below.

Establishment of additional bioassay activities is dependent upon availability of additional resources. At the current level of support we can introduce approximately 120 compounds per year into test systems. These are long-term (lifetime) bioassays and depending on the model used require an average of two years to complete. The American Chemical Society, Chemical Abstracts Service, indicates that approximately 200,000 new compounds come into existence each year. If, as has been estimated, approximately 1% of these become, through use, production, and distribution, a potential hazard to man, then some 2,000 chemicals should be tested every year. On this basis alone, the current level of effort is woefully inadequate. An attempt to partially resolve this problem is underway and is concerned with development of criteria for priority ranking, selection, and entry into the available test systems. These criteria will include (1) epidemiologic incrimination, (2) production, distribution, and use data, which may indicate the introduction of new compounds or new uses for existing compounds, (3) data on direct exposure to man, particularly ingestion, inhalation and body contact, and (4) structural relationship to known carcinogens. An information system is being developed for this purpose (Contractor: Stanford Research Institute).

(a) *Food Constituents, Additives and Contaminants* - Studies are being conducted to determine the carcinogenicity of a variety of substances in these categories. Among those currently under study are non-nutritive sweeteners, flavoring agents, preservatives, oxidized fats and essential minerals (several contractors: see Table II).

Extensive studies are conducted on mycotoxins, particularly aflatoxins, identifying their presence, chemical structure, toxicity and high carcinogenic activity, under different conditions. Aflatoxin levels were determined analytically in a variety of food products, from different areas, in an attempt to correlate these levels with epidemiological evidence of different incidences of cancer in man (Contractors: Massachusetts Institute of Technology and City of Hope).

A large bioassay operation on N-nitroso compounds is under way. It has already shown that most compounds in this chemical class are potent carcinogens affecting a variety of tissues and organs. The problem of the formation of these carcinogens in the organism by ingestion of nitrites and secondary amines has been the object of a series of bioassays in mice and rats. Preliminary results, using the induction of lung adenomas in mice as an index of activity, have suggested that the carcinogenic effect can be obtained by feeding nitrites and certain secondary amines separately (Contractor: University of Nebraska).

Importance: Regulatory requirements impose a zero tolerance for carcinogens in food. Recent reviews of the evidence have indicated a major inadequacy in the "safety testing" of food additives (e.g., compounds "generally recognized as safe" such as cyclamates, which have been proven to be carcinogenic). It is important to gain much more extensive knowledge of the possible carcinogenic hazards resulting from food intake, since they provide exposure to a vast segment of our population.

Naturally-occurring mycotoxins are widespread contaminants of foodstuffs stored under conditions of high temperature and humidity. The correlation between intake of these highly potent carcinogens and cancer in man is studied as a means toward the prevention of cancers that are highly prevalent in certain geographic areas (e.g., liver cancer in parts of Africa and Southeast Asia).

Nitrosamines are being studied with increasing emphasis because, they represent a class of chemicals consisting largely of potent carcinogens affecting a number of organs in all tested species. This is important because these compounds may be widely distributed at low levels in a variety of natural products and because they may be formed by the interaction in the body of nitrites with a variety of secondary amines present in food. Their target specificity for different organs makes this class of chemicals highly suspect as a major health hazard.

Future Plans: Further bioassay studies and efforts to identify occurrence and concentration in foods of recognized carcinogens. Additional effort will be directed to assessment of organ specificity and distribution of selected members of these groups of chemicals.

(b) *Air Pollutants* - A series of polynuclear hydrocarbons and related heterocyclic compounds found in polluted atmosphere (as well as in tobacco smoke) have been tested by direct administration into the respiratory tract of hamsters. While several tests are still under way, 7-H-dibenz[*c*,*g*]-carbazole has already proven to be a very potent respiratory carcinogen, capable of inducing squamous cell bronchogenic carcinoma at a much higher rate than benzo[*a*]pyrene (Contractor: University of Nebraska).

Several environmental air borne dusts (e.g. asbestos and metal oxides) are being tested by inhalation. In some cases these studies involve combined exposures with tobacco smoke. The complex tooling up phase has been completed and tests are now under way (Contractor: Battelle Northwest). A number of other bioassays, relevant to air pollution control are conducted in the context of the program of lung cancer (see below, [Contractors: IIT Research Institute, University of Nebraska, New York University, AEC--Oak Ridge National Laboratory, Ohio State University and University of San Francisco]).

Importance: Exposure to atmospheric pollutants is considered to be an important contributor to the total lung cancer hazard of the population: their definition and the study of their effects alone or in combination with tobacco smoke will be essential in the development of lung cancer prevention program.

Future Plans: Continuation of the program.

(c) *Tobacco and Smoking Products* - This portion of the bioassay program was developed with the expert advise of the Tobacco Working Group, established in 1968, under the chairmanship of the Scientific Director for Etiology, on the recommendation of the Lung Cancer Task Force. The task of this Group is to advise on the program aimed at the development of a less hazardous cigarette. A plan has been established following their recommendations. This activity is articulated in the following phases: selection, preparation, and characterization of different types of tobacco and tobacco smoke condensates for subsequent bioassay and analysis; bioassay of tobacco smoke and its fractions in a battery of animal systems; chemical analysis of the tobacco smoke and its fractions; and evaluation of the data obtained in relation to the human situation. A bioassay program has been developed to include preparation and analysis of different types of tobacco smoke condensates, bioassays on existing animal systems, particularly for topical carcinogenicity by mouse skin application, and for cytotoxic and ciliary-inhibitory effects on *in vitro* and *in vivo* systems.

Bioassays of over 20 types of cigarette smoke condensate are under way; skin tests already have started to show tumor induction by some condensates. Feeding tests and intratracheal instillation tests are in a preliminary phase. A major logistic operation has been established with the interlocking collaboration of several contractors. Major results of the skin tests are expected in the next fiscal year.

Inhalation tests using one type of cigarette are under way in one contract (Contractor: Battelle Northwest); their outcome will be critical in determining the feasibility of establishing a larger scale inhalation program. An improved inhalation device is being developed and tested through the collaboration of NIH staff with the AEC--Oak Ridge National Laboratory.

This program, supported by the special appropriation for the Lung Cancer Task Force, is closely correlated to the studies on respiratory carcinogenesis conducted in the Carcinogenesis Program.

Importance: There is overwhelming statistical evidence of a causal relationship between tobacco smoking and the occurrence of cancer of the respiratory tract in man: this type of cancer is responsible for the largest proportion of cancer deaths in men in this country. The development of a less hazardous cigarette is the only major alternative to individual cessation of smoking.

Future Plans: Continuation and expansion of this program to meet targeted goals.

(d) *Pharmaceutical Agents* - Several groups of drugs are being screened for carcinogenic activity. These include prescription and over-the-counter drugs, as well as restricted drugs (those anticipated for, or actually under, clinical trial). Special effort is being given to further evaluation of cancer chemotherapeutic agents, because many such drugs have application in the treatment of other diseases in which long-term survival is expected (i.e., the immunosuppressive drugs which have found increased use in organ transplant). Additional effort is being directed toward evaluation of those drugs which are being used for long-term or continuous therapy, such as the sulfonamides (rheumatic heart disease), isoniazid (tuberculosis) and the nitrofurans (chronic urinary tract infections).

The carcinogenicity of a variety of single agents or combinations of agents, mainly anticancer drugs in clinical use, is being tested in mice and rats. Preliminary findings indicate high incidences of benign and malignant neoplasia following treatment with several drugs, including Melphalan, Chlorambucil, uracil mustard, Natulan, dimethyltriazenoimidazole carboxamide, and 1,3-bis(2-chloroethyl-) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

Importance: Many drugs have never been adequately tested for carcinogenic activity. Knowledge of long-term toxicity hazards is needed for a meaningful evaluation of the balance of benefits and risks in the use of drugs.

Future Plans: Continuation of the screening of drugs and development of reliable criteria for the priority of selection for drugs which should be tested.

(e) *Industrial and Occupational Hazards* - Bioassay of a variety of chemicals which are primarily of industrial importance is underway, under several contracts (see Table II). Of these agents some are also air pollutants, food contaminants, or fall in the more general category of other environmental hazards.

Carcinogenicity of the following compounds has been observed in feeding tests: propylene imine, propane sulton and ethylenethiourea (Contractor: Bionetics Research Laboratories); o-toluidine and 4-chloro-o-toluidine (Contractor: Bio-Research Consultants); topical carcinogenicity at the site of injection was shown for propane sultone, aziridine and seven other compounds (epoxides, lactones, and derivatives [Contractor: New York University]).

Studies are underway to determine the carcinogenicity of various metals, metallic compounds and their derivatives. Results to date indicate a carcinogenic response in animals to certain compounds of nickel, cadmium, titanium, cobalt, zirconium, chromium, and to a nickel-copper alloy. The compounds have been first tested by intramuscular injection in rats, and tests are now underway by the oral and respiratory routes.

Importance: Identification of risks resulting from occupational exposure or from population exposure to industrial chemicals is an important step in a program of environmental cancer prevention. In some cases, industrial compounds become broadly disseminated in the environment. For example, titanium dioxide, carcinogenic by injection, has recently become a common ingredient of household paints. Any increased risk to cancer, attributable to industrial or occupational usage is likely to be among those risks which would lend themselves to reduction or elimination through currently available methods.

Future Plans: To continue the studies underway and to identify additional chemicals or compounds which should be tested.

(f) *Pesticides* - After completion of the first and only large scale carcinogenesis screening project for pesticides, in previous annual reports, a new series of long-term tests has been set up for a second level testing in two species of laboratory animals. Extensive chemical analysis has been performed and the test compounds have been characterized and tested for acute toxicity. Long-term bioassays have been initiated (Contractors: Gulf South Research Institute and Hazleton Laboratories). Other tests of pesticides are continuing under several contracts (see Table II).

Importance: The wide distribution of pesticide chemicals in the environment makes it important to identify those among them which represent potential long-term hazard to man. The NCI studies are important in generating new national policies on the problem of pesticide control, and in helping to meet the essential need for properly planned, well-controlled, large-scale bioassay programs in this field.

Future Plans: Performance of the second level testing of pesticides, as indicated by the screening program and continuation of screening bioassays.

(g) *Other Environmental Hazards* - A variety of chemicals, representing different categories of potential human exposure, are under test. Preliminary results were obtained showing the potentiation of skin carcinogenesis by certain optical brighteners combined with exposure to low wavelength ultraviolet light (Contractor: University of Cincinnati); these studies are being extended with further control of the experimental conditions.

Certain mycotoxins, derived from fungi which are common dermatological pathogens in man, have been extracted, analyzed and screened for carcinogenicity. Preliminary results indicate that carcinogenic activity is present in some extracts (Contractor: Temple University).

Several substituted hydrazines have been shown to be carcinogenic in mice and to have a characteristic organ-specificity dependent on their chemical structure (Contractor: University of Nebraska).

Importance: Bioassay screening of chemicals from various environmental categories is important for identifying new areas of concern in the attempt to monitor the environment of cancer hazards.

Future Plans: Development of these studies.

5. *Development of Biological Models*

Studies designed to develop and define new animal models for carcinogenesis are becoming the focus of one of the major portions of the Carcinogenesis Program.

A major component of this effort is the Special Lung Cancer Program, which has developed in the past two years. A program on colon carcinogenesis is being developed. Other special programs on bladder cancer, endocrine-related cancers, childhood cancers and pancreas cancers have been considered for implementation as soon as personnel and resources become available.

(a) *Special Lung Cancer Program* - A complex effort is directed towards the definition of causative factors and the development of preventive measures for cancer of the respiratory tract. Lung cancer represents a major cause of death in the United States, now estimated to reach 64,000 deaths per year. This program is closely correlated with the tobacco bioassay efforts.

The Lung Cancer Unit, OASDC, has reached an adequate staff level in this fiscal year and has become a major contributor to the scientific advancement of the lung cancer program.

The major obstacle to the development of a direct attack on this problem in the past was represented by the lack of experimental models capable of reproducing this type of cancer in animals by treatments related to the conditions of exposure in man. Recently, experimental models have been developed primarily through support by the NCI Carcinogenesis Programs. These research accomplishments have made it possible to develop a new program concentrated on lung cancer prevention.

Further progress has been made during the last year in defining biological animal models that would adequately reproduce the main features of development of bronchogenic carcinoma as seen in man. An articulated group of bioassay systems has been derived from these observations and is now being submitted to a process of standardization in animal studies aimed at developing adequate bioassay models for respiratory carcinogens, including tobacco smoke (several contracts: see Table II).

Since particulates appear to play a significant role in respiratory carcinogenesis, production and physico-chemical characterization of defined particulate materials has been established to identify the parameters responsible for the biological response. Extensive biological experiments are under way. Current findings confirm that particle size plays an important role in the retention of carcinogens in the lung (Contractor: IIT Research Institute).

Methods have been developed for the separation of respiratory epithelium from underlying connective tissue and cartilage and for preparing metabolically active subcellular particles, such as nuclei, from the epithelial cells (Lung Cancer Unit, OASDC). This methodological advance has enabled us to develop an experimental approach to the definition of morphological-biochemical correlations directly in the target cells of respiratory carcinogens. This approach could lead to the identification of critical changes which could then be studied in human respiratory epithelia. In this way it is planned to attempt to identify key processes in the pathogenesis of lung cancer that can become the target for inhibition, and also identify critical tissue markers that can be used to recognize highly susceptible individuals or predisposing conditions.

Methods have been developed for the use of short-term organ cultures of tracheobronchial segments to determine metabolic activities in the respiratory epithelia following *in vivo* and/or *in vitro* exposure to carcinogens, inhibitors and indicators of molecular control mechanisms. Morphological, ultrastructural and biochemical techniques are used for the definition of the critical events during carcinogenesis in the respiratory epithelium (Lung Cancer Unit, OASDC).

Organ culture studies using long term maintenance of tracheobronchial segments have been continued and extended to a preliminary observation of human specimens. The induction of atypical metaplasia by diethylnitrosamine, and its inhibition by vitamin A, in this *in vitro* system, suggests a cellular control mechanism independent of other organs and of the type of carcinogen used (Contractor: University of California at San Francisco).

Since vitamin A is required for normal differentiation of respiratory epithelium, and since it blocks the effects of carcinogens in this tissue, studies on the key cellular mechanisms controlled by retinol and related substances are actively pursued in the Lung Cancer Unit and under contracts.

A major achievement in the study of the control of mucus-squamous cell differentiation by vitamin A has been the discovery that retinol is necessary for the biosynthesis of a specific fucose-containing glycopeptide. An indirect immunofluorescence method was developed which specifically detects the cellular localization of this glycopeptide. This bears special significance to the problem of cell differentiation since it was found that both in intestinal and tracheal epithelia the number of goblet cells drastically decreases in vitamin A deficiency. Hence the immunofluorescence assay can be used as a marker for mucus cell formation. Moreover, it was found that the synthesis of the same glycopeptide was severely decreased in vitamin A deficient lung tissue. In a transplantable bronchogenic carcinoma of the hamster, the relative distribution of glycopeptides is drastically changed by administration of retinoic acid to the host, and growth of the tumor is markedly reduced (Contractor: Massachusetts Institute of Technology).

In the hamster model for intratracheal administration, now widely used in this Program, recent tests show the high carcinogenic activity of 7-H-dibenz-[*c*,*g*]carbazole and of dibenz[*a*,*i*]pyrene, two compounds found in air pollution and tobacco smoke and previously untested on the respiratory tract (Contractor: University of Nebraska). Addition results show that bronchogenic tumors can be induced by suspensions of benzo[*a*]pyrene in saline/gelatin even without carrier particles, although apparently at low incidences (Contractor: IIT Research Institute). A considerable amount of work is now under way on the role of particles in respiratory tract carcinogenesis with topical and systemic carcinogens. Cell kinetic studies are conducted to determine the specific cellular effects of the treatments: it was found that a hyperplastic response and a labelling peak are induced by ferric oxide suspensions even without carcinogen (Lung Cancer Unit, OASDC, and Contractor: Marquette University).

Large doses of polynuclear hydrocarbons in saline, administered intratracheally to rats and to mice have been shown to induce a high incidence of squamous cell carcinomas of the lung (Contractor: AEC--Oak Ridge National Laboratory). Similar treatment schedules have produced a very early neoplastic response in hamsters (Lung Cancer Unit, OASDC, and Contractor: Microbiological Associates, Inc.).

Carcinogenic effects for the respiratory tract of hamsters have been shown by systemic administration of dibutylnitrosamine (also a bladder carcinogen), as it was previously seen for diethylnitrosamine. It was also found that these two nitrosamines can be carried through milk and induce tumors of the trachea and nasal cavities in hamsters that have been nursed by carcinogen-treated females (Contractor: University of Nebraska).

Additional evidence of the carcinogenic effects of chromates has been obtained (Contractors: New York University and AEC--Oak Ridge National Laboratory). The induction of lung cancer by the industrial compounds of the halo-ethers group has been further documented (Contractor: New York University).

Importance: The whole program on lung cancer is reaching a very productive and exciting phase. Key carcinogens are being identified by bioassay, the specific patterns of target tissue response are being identified and in part successfully inhibited. The study of some critical molecular control mechanisms has become possible and is giving important results. The prevention of lung cancer in man can only in part be achieved by removal of causative factors in our society. It is essential therefore that a concerted effort be made to identify all possible targets for protective or inhibitory corrective measures at the host level. This effort is now fully under way.

Future Plans: Contination and development of this targeted program.

(b) *Special Colon Cancer Program* - This program is now in a planning stage and is correlated with the national effort undertaken under the aegis of the Colon Cancer Task Force. New contracts are being negotiated to start implementation of the program. Present in-house studies represent an important contribution to the development of this effort. It was shown that beta-glucuronidase from certain intestinal bacteria of hydrolizes carcinogen metabolites entering the gut as conjugates *via* the bile; metabolic splitting of the conjugates is followed by reabsorption of the products. Other enzyme systems present in the intestinal flora and active as modifiers of carcinogen metabolites are being studied (Experimental Pathology Branch).

The recently discovered colon carcinogen, 1,2-dimethylhydrazine, has been synthetized with the methyl groups labelled with ^{14}C; preliminary results suggest that this compound reacts as an alkylating agent in a manner similar to the carcinogenic N-nitroso compounds (Chemistry Branch with the cooperation of the Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, England).

The Epidemiologic Pathology Unit, recently transferred to OASDC from Demography, is also making a major contribution to this program.

Importance: Colon cancer is one of the major forms of cancer in man. Sufficient new leads are now available to support a comprehensive targeted program, which is being initiated.

Future Plans: To develop a coordinated program.

(c) *Other Studies of Biological Models* - A variety of studies is under way to increase the effectiveness of our biological tools in carcinogenesis studies. Some notable results in the current year include the following:

(1) In liver carcinogenesis, methylcholanthrene was found to increase the incidence of carcinomas and of cirrhosis in rats given carbon tetrachloride. It was also found that undifferentiated liver carcinomas usually do not metastasize (because the host dies rapidly): if the carcinoma is excised surgically, the host lives longer and metastases develop. In the mouse, spontaneous liver carcinomas are easily transplantable, while liver nodules diagnosed as hyperplastic lesions do not survive on transplantation (Experimental Pathology Branch). The biochemical and growth characteristics of hyperplastic liver nodules induced by aflatoxin have been further defined and found to correspond to those of nodules induced by other different carcinogens, suggesting that early biochemical lesions, may become useful markers of carcinogen exposure (Contractor: Temple University).

(2) Induction of a high incidence of ovarian tumors in the hamster by intravenous administration of the carcinogen, 7,12-dimethylbenz[*a*]anthracene (Contractor: University of Nebraska); this may become a choice model for the study of this important type of endocrine-related cancers.

(3) The role of age of the host in determining the neoplastic response has been demonstrated more dramatically than even before by the results of the administration of the carcinogen ethylnitrosourea to newborn or infant mice. They developed over 20 different types of tumors in almost every major organ and tissue, in comparison to young adult mice of the same strain, which only developed four types of primary tumors (Contractor: University of Chicago). This model system is being defined and standardized for use in bioassays.

(4) Studies on the transplacental passage of carcinogens and the induction of tumors in the offspring of treated mothers have been continued (Experimental Pathology Branch): a program of studies on models for childhood cancers is being developed.

Importance: The definition of the biological response to carcinogens, specific to each major organ system, represents a major need in the development of coordinated attacks to the principal types of cancers seen in man.

Future Plans: To extend studies to other models for the major forms of cancer seen in man.

(d) *In Vitro Models for Carcinogenesis* - Study of *in vitro* systems for chemical carcinogenesis has been considerably expanded in this year. Major advances have been made in several intramural laboratory programs and by a number of contract supported projects.

Transformation of hamster embryo cells has been accomplished with at least 15 chemical carcinogens of different chemical types; their *in vivo* and *in vitro* activities appear well correlated. Transformed clones reimplanted *in vivo* have been shown to give rise to malignant tumors (Biology Branch). Urethane and diethylnitrosamine, which require metabolization to become active carcinogens, were negative when tested *in vitro*; but when administered transplacentally to the hamster embryo from which cell cultures were made were capable of inducing high incidences of transformation in the cultured cells without further *in vitro* treatment (Biology Branch).

A remarkable enhancement of transformation was found by combined effect of X-irradiation and chemical carcinogens (Biology Branch).

The same hamster embryo cell system is used in studies by the Chemistry Branch in collaboration with Dr. Sach's laboratory at the Weizmann Institute. In these important studies, this contractor has found that cells transformed by benzpyrene and dimethylnitrosamine can produce a high frequency of variance with a significant reversion of properties of the transformed cells. These variants lack the pattern of growth exhibited by the parental transformed cells and reverted to a contact inhibition of cell replication similar to that exhibited by normal cells. The variants showed a decrease in tumorigenicity, saturation density, and cloning efficiency. They also acquired a limited lifespan *in vitro*. These important results have shown that cells that are transformed by carcinogens can, under appropriate conditions, revert back to normal cells. These results are of such major importance that additional confirmatory studies are being planned. If confirmed, these results represent a major change in our understanding of the nature of tumor cells (Contractor: Weizmann Institute of Science).

Another *in vitro* system developed at New York University has been shown to respond with an altered growth pattern to promoting agents, and is being further investigated (Contractor: New York University).

Several projects are devoted to the very critical issue of developing *in vitro* systems for the transformation of epithelial cells.

The methods developed in the last two years for the culture and transformation of mouse skin cells, have shown that the carcinogen, 7,12-dimethylbenz[*a*]-anthracene and the promoting agent Tween 80 induce morphologic transformation, indefinite growth of the cell lines and tumors when the transformed cells are implanted *in vivo*. Morphologic criteria suggest that the cells retain their epithelial character throughout (Experimental Pathology Branch). This may be considered the first demonstration of *in vitro* neoplastic transformation of epithelial cells.

Stable epithelial rat liver cell lines have been cultivated *in vitro* for over a year in the absence of spontaneous transformation. A number of carcinogens induce transformation in these lines; however, so far, injection of the transformed cells into syngeneic hosts failed to give rise to visible tumors (Experimental Pathology Branch).

Organ culture systems have been developed for specific tissues. Those for respiratory tissues have been described above (see Special Lung Cancer Program). A system of *in vitro* culture of mammary tissue is being used for studies of the effect of hormones on RNA metabolism in this tissue (Chemistry Branch).

Importance: The development of *in vitro* models of chemical carcinogenesis is an important breakthrough that will lead to the possibility of short-term carcinogenesis testing on selected target cells and on the other hand, to the possibility of identifying key cellular control mechanisms of carcinogenesis in well-defined biological systems.

The demonstration of transplacental activity of carcinogens on embryo cells which are then observed *in vitro* is very important because it establishes the possibility that some of the so-called "spontaneous" transformations observed in culture may be due to chemical carcinogens reaching the target cells *in utero*.

The demonstration of reversion of chemically-transformed cells may open the way to a major advance in the understanding of the control of tumor growth.

The advances in the demonstration of epithelial cell transformation by chemicals are important for our development of selective target cell systems applicable to the determination of susceptibility of human cells to carcinogens.

Future Plans: Expansion of these programs with emphasis on chemical-viral interactions.

6. *Identification of Carcinogenesis Processes*

Various processes involved, or thought to be involved, in the mechanism of transformation or tumor induction are being investigated. Among these are the establishment of criteria for distinguishing between carcinogens and non-carcinogens, the discovery of steps in the process of carcinogenesis that may be susceptible to inhibition and the development of diagnostic tests and therapeutic procedures for cancer based on host reactions to carcinogenesis. Additional studies underway include investigations on the nature of the interaction, on the nature of the interaction between carcinogenic agents with living organisms at the host, tissue, cellular and molecular levels and to clarify the functional consequences of these interactions at each level.

An increased effort has been made to develop specific correlations between the identification of key steps in the carcinogenic processes and their applicability to the control of induction of cancer in selected organ systems for which adequate biological models have been developed.

Some of the highlights of progress in this complex part of the Program are listed below under headings corresponding to the major steps of carcinogenesis as identified in the Program Plan. More detailed findings are given in the Branch Reports and in the contract narratives.

(a) *Penetration of Carcinogens* - Further studies on the penetration of carcinogens in the respiratory tract have indicated major differences in the penetration and retention of polynuclear hydrocarbons in the respiratory tract, depending on particle size and surface aggregation (see Special Lung Cancer Program). New studies have been developed on the penetration of carcinogens through the intestinal mucosa in relation to colon cancer (Experimental Pathology Branch).

Importance: Defining the conditions for effective entry of carcinogens into the organism or the target tissues is a basis for attempting to minimize exposure.

Future Plans: Further definition of the essential requirements for carcinogen penetration in key target tissues.

(b) *Distribution and Metabolism of Carcinogens and Cellular Factors Responsible for Metabolic Activation* - Considerable further progress has been made towards the elucidation of metabolic pathways and activation of different classes of carcinogens.

For polynuclear hydrocarbons, studies on the microsomal enzyme system responsible for their metabolism have been further developed. The distribution of the enzyme has been determined in several species, strains, and tissues. Its molecular control has been studied in depth. Over 20 compounds have been screened for their activity on the enzyme, and inducers as well as inhibitors have been identified. The requirement of this enzyme for polynuclear hydrocarbon toxicity and binding to nucleic acids has been shown. A potent enzyme inhibitor, which also strongly inhibits the carcinogenic activity of dimethylbenzanthracene has been discovered (Chemistry Branch).

The role of microsomal enzymes in the toxicity and transformation by polycyclic hydrocarbons has been studied by collaboration with the Weizmann Institute. Various inhibitors of the enzyme have been found to prevent *in vitro* neoplastic transformation by benzpyrene. This suggests that the metabolism of benzpyrene is required for cell transformation as well as cytotoxicity (Contractor: Weizmann Institute of Science).

The metabolism of benzo[*a*]pyrene leading to its critical activation and interaction with target cell constituents has been further studied (Chemistry Branch).

For liver carcinogenesis, the enzymatic control of metabolic activation of N-2-fluorenylacetamide was further defined. It was also found that 8-hydroxyquinoline delayed tumor formation by this carcinogen and severely inhibited liver carcinogenesis by diethylnitrosamine (Experimental Pathology Branch).

A key discovery, reported in a preliminary way last year, has been confirmed and documented: sulfate esters of the N-hydroxy derivatives of aromatic amines are the significant ultimate carcinogens for liver cancer induction from aromatic amines. An elegant demonstration of this key step in carcinogen activation has been given by showing that an excess of acetanilide, a compound which produces a depletion of endogenous available sulfate, inhibits toxicity and carcinogenicity of the precursor carcinogen, while an increase of the dietary sulphate level to a level equimolar to that of the acetanilide or higher, restores the carcinogenicity (Experimental Pathology Branch).

Further studies on the metabolism of liver carcinogens are continuing: several inhibitor systems have been identified and the conditions of their effectiveness studied (Experimental Pathology Branch).

It has been shown that the ability of the rat liver, but not of the kidney, to metabolize dimethylnitrosamine is decreased by a protein deficient diet. As a result, more of the dose of carcinogen is metabolized in the kidney and the site of tumor induction is shifted from liver to kidney (Chemistry Branch).

Another significant advance was the discovery of a new metabolite of aflatoxin, and the development of a method for its analysis. This metabolite, an O-dimethyl derivative, is excreted in the urine. Thus, it may be possible to determine human consumption of aflatoxin by measuring excretion of the metabolite and thereby relating the eventual occurrence of disease to the consumption of aflatoxin (Contractor: Massachusetts Institute of Technology).

Importance: The identification of the metabolic pathways essential for the action of carcinogens represents a key step towards the goal of inhibiting carcinogenesis at the host level. The definition of the essential metabolic control mechanisms for polynuclear hydrocarbons is a very important tool for the identification of susceptibility of individuals to these carcinogens that have a prominent role in the induction of cancers of the lung and of the skin.

Similarly, the definition of the key activation steps for aromatic amine carcinogens is highly relevant to our understanding of the exploitable mechanisms for the prevention of cancers resulting from a number of environmental, occupational and dietary carcinogens. Elucidation of such key metabolic steps is also an essential requirement for comparing the response of man to that of animals to the same carcinogens. Comparative metabolic studies will be one of the critical keys for an evaluation of risks in man following exposure to environmental chemicals known to be carcinogenic in animals. This is an area of great importance for the development of public health and regulatory criteria.

Future Plans: Extension of these studies and of their applicability to man.

(c) *Interaction of Carcinogens with Cell Constituents and Neoplastic Transformation* - A complex series of studies is devoted to these key problems in carcinogenesis.

Most of the present emphasis is on the definition of the nature of the interaction of carcinogens with nucleic acids and the role of these interactions on the control of cellular function.

Major methodological advances continue to be made in the techniques for isolation and analysis of nucleic acids.

The study of RNA's found in the nucleus has been made much more meaningful by recent improvements in the way in which nucleic acids are isolated and analyzed. The technique of gel electrophoresis can be successfully employed on small samples which can be prepared rapidly, thus minimizing the possibility of loss of labile forms. Using this analytical approach it has been found that there are two new classes of RNA of high molecular weight, approximately 5 and 10 million daltons.

It has been possible to show that these forms are not ribosomal precursors, and hence must play a different role in the cellular physiology (Chemistry Branch).

The same experimental approach has shown that DNA may be studied in an analogous way. It is possible to differentiate between main band and satellite DNA electrophoretically, and this approach has been used to study the possibility of differential binding of carcinogens to these fractions of nuclear DNA. In studies complete to date, there has been no differential binding (Chemistry Branch).

Studies are near completion on the role of molecular conformation in determining the electrophoretic properties of polynucleotides in agarose-acrylamide gels. These important studies have indicated that it will be possible, with very small amounts of biological material, to determine whether a newly isolated polynucleotide (e.g. from a new virus) is RNA or DNA, single- or double-stranded, circular or linear. Similar determinations should be possible on newly found polynucleotides isolated in the presence of large amounts of normal cellular nucleic acids. These studies will be of utmost importance in clarifying the nature of changes in RNA and DNA during tumorigenesis (Chemistry Branch).

Electron microscopic observation of the molecular organization of RNA in several RNA viruses has been conducted; the site of DNA synthesis induced by murine sarcoma virus has been studied by electron microscopic autoradiography (Chemistry Branch).

Earlier studies in the Chemistry Branch demonstrated a requirement for new DNA synthesis during the early stages of infection by RNA tumor viruses. This demonstration and the finding that no new protein synthesis was necessary for the synthesis of this DNA led directly to the recent discovery of RNA-dependent DNA synthesizing enzymes in RNA tumor viruses. The presence of these latter enzymes established a biochemical rationale for viral RNA acting as a genetic template for the synthesis of viral DNA. The use of 5-bromodeoxyuridine (BUdR) provided genetic support for an intracellular viral DNA which acts as the replicative form for the virus. Lethal and sublethal mutations were found after incorporation of BUdR into DNA during early stages of infection. Noninfectious, virus particles were produced containing intact but presumably defective viral RNA, and temperature-sensitive (conditional lethal) mutants were isolated from virus populations produced after exposure of cells to BUdR. The most likely interpretation of these data is that a faulty (BUdR-containing) DNA is synthesized complementary to the infecting viral RNA, that this DNA is transcribed to make progeny viral RNA, and in the process of this transcription defective viral RNA is synthesized (Chemistry Branch).

Important developments have occurred in the study of DNA repair mechanisms and their role in the cellular control of carcinogenesis. A new and sensitive assay for excision repair of DNA has been developed; this assay yields the numbers, sizes and distribution of repaired regions (Contractor: AEC--Oak Ridge National Laboratory). It was also shown that transforming bacterial DNA, treated with benzo[α]pyrene, can be partially reactivated by bacteria able to repair UV-damaged DNA; mutant bacteria, lacking the latter capacity, are also deficient in repairing DNA damage induced by chemical carcinogens (AEC--Oak Ridge National Laboratory with the cooperation of Dr. P. O. Tso, Johns Hopkins University).

The effect of carcinogens on the function and fate of nucleic acids is also studied in animal models for tumor pathogenesis. A single dose of N-methyl-N-nitrosourea decomposes in the rat within 15 minutes after administration, yet this dose is sufficient to produce cancer in almost every treated animal. In this model system, analysis of the macromolecular structure of both DNA and RNA is carried out by both gel electrophoresis and zonal centrifugation. Results already obtained show that incorporation of precursors is affected earlier and more severely for DNA than for RNA. Preliminary results also indicate that this carcinogen may lead to fragmentation of DNA *in vivo* (Chemistry Branch): if confirmed, these observations could suggest a major new pathway of carcinogen action on nucleic acids.

Further experiments were conducted to determine the conditions of DNA binding of the polynuclear hydrocarbon carcinogen, 7,12-dimethylbenz[*a*]anthracene (DMBA) in skin cells in culture. Further refinements of techniques were used; the results confirm that the carcinogen DMBA can bind to non-replicating as well as to replicating DNA; that DNA to which DMBA is bound is capable of subsequent replication; and that in such replication all of the hydrocarbon is found to be bound to the parent strand with no detectable binding to the daughter strand. These experiments reinforce the previous experiments indicating that the binding of DMBA to DNA does not prevent replication, that the DMBA binds mostly to a single strand of DNA with little if any cross-linking, and that the DMBA remains bound to the DNA during the process of replication. Current investigations are designed to determine whether the presence of the DMBA on a replicating molecule of DNA has any effect on the structure of that molecule of DNA or on its subsequent function (Experimental Pathology Branch).

Work on transfer RNA has continued, in view of the potential importance of this cellular control system for carcinogenesis; examination of tRNAs of various organs has revealed important differences in seryl-tRNA patterns. An especially interesting finding is the presence of a type of seryl-tRNA which recognizes a codon (UGA) not used for protein synthesis in bacterial systems (Chemistry Branch).

Methylation of nuclear RNA is being studied with improved techniques in rat liver and in the respiratory tract epithelium. Exoribonuclease has been identified in the nucleus of tracheal epithelial cells (Lung Cancer Unit, OASDC). Inhibitors of this enzyme have been developed and synthesized; the goal of selectively inhibiting RNA degradation without inhibiting RNA synthesis has been met in an *in vitro* situation (Contractor: Ash Stevens, Inc.).

Considerable advances have been made in the field of *mutagenesis* towards the demonstration of a correlation between carcinogenic and mutagenic activity of chemicals (Contractor: AEC--Oak Ridge National Laboratory). The following summary is from the report of the Oak Ridge group:

> Mutagenicity of carcinogens is being evaluated with test systems capable of detecting all types of genetic damage, some of which can be characterized at the molecular level, and particular attention is paid to assaying mutagenicity of metabolic derivatives (proximal and ultimate carcinogens) as well as the parent compounds. This approach is beginning to show a high correlation between carcinogenic and mutagenic activity, and also between carcinogenicity and a particular type of mutagenic activity. In general, it can be said that a high percentage of the mutations caused by carcinogens are those that would produce a gene product of low activity rather than the partial or complete absence of the gene product.

A variety of types of precarcinogens, proximate, and ultimate carcinogens were tested in the Neurospora system in FY 1971. Development of methods for the production of ultimate carcinogens in the mutation assays has continued. These include chemical hydroxylation systems, microsomal enzymes, and host-mediated assays, and are being applied in mutation assays with Neurospora, hamster cells, and with Salmonella. In the latter system the carcinogen DMN was highly mutagenic under conditions favoring chemical hydroxylation but not when hydroxylation was prevented. Carcinogenicity of DEN in Neurospora has been similarly examined with comparable results.

A host-mediated assay system for mutagenicity testing with Chinese hamster cells has been developed. In preliminary experiments it was found that there is a significant increase of spontaneous mutation frequency in cells sequestered in the abdominal cavity of a mammalian host. Similar results were found previously with Neurospora treated in this fashion.

A major effort was made to develop new assays for detection of gene mutations in Chinese hamster cells, preparatory to a planned effort to utilize these cells to develop a system in which *in vitro* carcinogenesis can be analyzed in cells of known genetic background and wherein the genes can be experimentally manipulated.

A number of studies have direct relevance to chemical-viral interactions in carcinogenesis; much new work has been undertaken this year in this important field, partly in collaboration with the Viral Oncology Area and through contracts.

Importance: The whole problem of the interaction of carcinogens with cell constituents is critically important in the identification of exploitable mechanisms of carcinogenesis.

Future Plans: Development of these studies with emphasis on their applicability to specific organ systems.

(d) *Growth Control and Immunological Factors* - A number of the studies reported above also have a direct bearing on the control of cell growth following neoplastic transformation. One major area of interest in this field is represented by the relationships between cellular differentiation and carcinogenesis with their respective molecular control mechanisms. An important goal in the Program is to determine how much there is in common between the control of differentiation present in normal cells and the altered control of differentiation that leads to the expression of a tumor cell. Work along these lines is actively pursued in our model systems for respiratory carcinogenesis, where mucous-to-squamous differentiation is intimately related to the normal-to-neoplastic conversion in response to the same agents and inhibitors. This work has been discussed in the report of the Lung Cancer Unit, OASDC.

Work on *in vitro* systems, such as those described above in the report on Biological Models, also bears direct relevance to this problem.

Experiments involving manipulation of cell density demonstrate that transformation of an infected cell by Rous sarcoma virus can be influenced by neighboring cells. Such phenotypic suppression of malignancy may be important in tumorigenesis and is being further investigated (Chemistry Branch).

A major component of our Carcinogenesis Program concerns the definition of the role of immunological mechanisms in the development of carcinogenic responses and in the control of tumor cell growth.

An active program on immunological mechanisms in carcinogenesis has been under way at the Oak Ridge National Laboratory. Most of it is now supported by contract funds from the Viral Oncology Area.

Studies related to chemical carcinogenesis are being developed to correlate both the histopathologic aspects of the systemic immune response and anatomic factors which involve progressive growth of chemically induced tumors with functional immunologic factors directed against success of the tumor.

An extensive program on tumor immunology has been actively developed in the in-house laboratories of the Biology Branch, and is now being expanded through contract-supported collaborative projects.

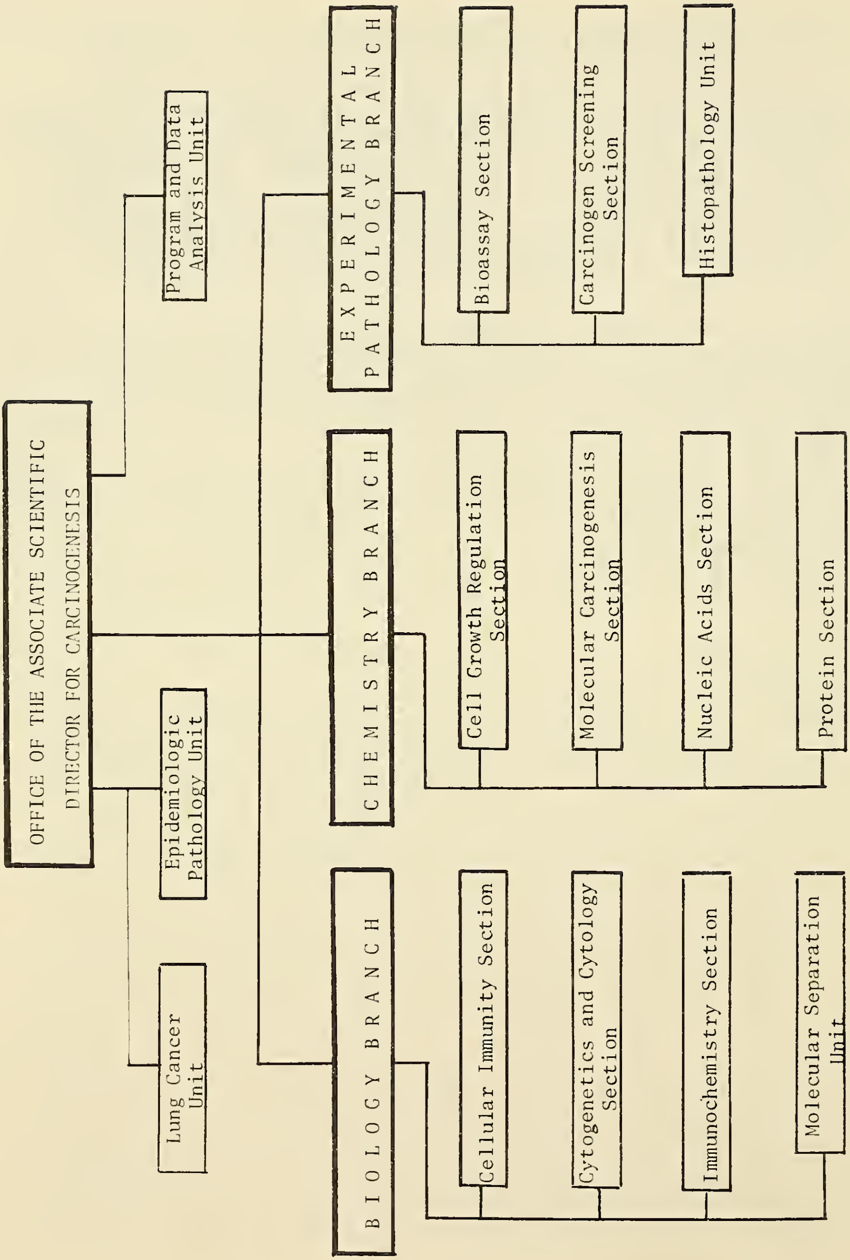
Progress highlights in this Biology Branch program are summarized as follows:

> Advances have been made in the isolation and characterization of normal and malignant cell surface antigens, in methods for the control of tumor growth *in vivo* and in the role of humoral factors in carcinogenesis. A method that appears to have applicability to the extraction of surface antigens from a wide variety of cells, has been used to solubilize tumor specific antigens from transplantable diethynitrosamine-induced guinea pig tumors. This method is now being applied to the extraction of antigens from human tumors. The growth of apparently non-antigenic or weakly antigenic tumors is suppressed in animals immunized with mixtures consisting of living Mycobacterium Bovis (strain BCG) and living tumor cells. Furthermore guinea pigs with palpable intradermal tumors and regional lymph node metastases can be cured by the injection of BCG directly into the intradermal tumor.
>
> Members of the Biology Branch have published a book entitled *Molecular Basis of Complement Action*. This book contains a description of much of the complement research carried out in the Biology Branch. Macrophages have been found to be capable of *in vitro* synthesis of the second (C2) and the fourth (C4) components of complement. These components are being used as biochemical markers in transformed and untransformed cell cultures. A new, extremely sensitive and accurate method for the detection of bound

C3 (the third component of complement) has been developed. This method will be used to study the role of circulating antibodies in the carcinogenic process. In analyzing the mechanism of cell destruction by antibody and complement we have uncovered an amplifying mechanism whereby a single antigen-antibody complex on the cell surface after interaction with complement can generate multiple cytolytic sites. About two weeks after beginning chronic administration to guinea pigs of a non-toxic, carcinogenic dose of diethylnitrosamine, the serum content of the fourth component of complement (C4) is reduced to less than 5% of the normal level; none of the other eight components is significantly effected.

Importance: The development of growth control mechanisms in the inhibition of carcinogenesis is one of the major targets for the Carcinogenesis Program. It is particularly important to determine how the wealth of scientific information and the methodologies developed by basic studies in immunology can be brought to bear on the inhibition or control of carcinogenesis.

Future Plans: Extension of these studies, particularly through the contract mechanism, and application to specific carcinogenesis models in animals and to cancer prevention in man.


OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS
Lung Cancer Unit
Epidemiologic Pathology Unit
Program and Data Analysis Unit
BIOLOGY BRANCH
Cellular Immunity Section
Cytogenetics and Cytology Section
Immunochemistry Section
Molecular Separation Unit
CHEMISTRY BRANCH
Cell Growth Regulation Section
Molecular Carcinogenesis Section
Nucleic Acids Section
Protein Section
EXPERIMENTAL PATHOLOGY BRANCH
Bioassay Section
Carcinogen Screening Section
Histopathology Unit

II. *OFFICE AND BRANCH REPORTS*

Summary Report

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

July 1, 1970 through June 30, 1971

The direct operation of the Office of the Associate Scientific Director for Carcinogenesis includes the immediate office of the Associate Scientific Director for Carcinogenesis, the Program and Data Analysis Unit (J. A. Cooper, II, Head), the Lung Cancer Unit (M. B. Sporn, Head), and - since March 1971 - the Epidemiologic Pathology Unit (J. W. Berg, Head). The report of the activities of the latter Unit is given under the Summary Report of the Demography Area.

The planning and coordination of the whole Carcinogenesis Area program has continued to take the major effort of this office, now most effectively assisted by the development of special monitoring systems in the Program and Data Analysis Unit. Dr. Allen H. Heim has joined this Office as Coordinator of Collaborative Research and is instrumental in developing a new management structure for the Carcinogenesis Program. An Interim Collaborative Program Review Group has been established for relevance, need and priority review of Carcinogenesis contracts. Mr. Ray Newell of this Office has assumed responsibility as Executive Secretary of this Group and of the Chemical Carcinogenesis Contract Review Committee.

This Office has continued to serve as the focal point for the development of documentation on the assessment of environmental carcinogenesis hazards and on the principles for evaluation of environmental carcinogens, in collaboration with the Biometry Branch, Demography, Etiology, and with the Office of the Director, NCI. In this capacity this Office has participated in a number of review and policy meetings at the request of other government departments and agencies, including: The Office of Science and Technology, Executive Office of the President; Committee on Chemicals and Health, President's Science Advisory Committee; Subcommittee on Pesticides, President's Cabinet Committee on the Environment; Office of the Surgeon General, USPHS; HEW Departmental Pesticide Coordinating Committee; Groups on Recommendations for National Center for Toxicological Research, Office of the Secretary, DHEW; several committees and ad hoc groups with FDA, EPA, USDA and other agencies.

Statements were prepared for testimonies given by the Associate Scientific Director at the hearings of two Senate Subcommittees, respectively on Pesticides and on Chemical Carcinogenic hazards.

This Office also sponsored a Working Group on Artificial Sweeteners, a Workshop on the Penetration and Uptake of Nucleotides into Animal Cells, and a Workshop on the Identification of Agents Carcinogenic for Man.

These and other related activities in the area of evaluation of environmental hazards have required a major share of the resources of this office, including communication with Congressional Committees, other Government agencies, the press, and the Information Officers at NCI, NIH, and DHEW. Major emphasis has been given in all these activities to the unique role of NCI in providing documentation of carcinogenesis bioassay studies and scientific criteria for evaluation of carcinogenic hazards for man.

Program and Data Analysis Unit: The activity of the Program and Data Analysis Unit has provided the Associate Scientific Director with staff support in data processing, program planning and evaluation, and development of collaborative activities. Its members have also continued to manage a number of contracts, particularly those dealing with chemical and information resources.

Initial development of the Carcinogenesis Bioassay Data System has been completed. The system is being submitted to a pilot test by selected contractors performing bioassay studies and is expected to reach full operation in FY-72. The Carcinogenesis Chemical Information System contains an ever-growing file of data on materials presently under test or projected to be under study. The Carcinogenesis Contract Data System continues to operate as a central source of administrative data on the elements of the extramural program.

Through contracts managed by the Unit, PHS Publication No. 149 supplements, covering literature of 1961-64 and 1968-69, will be in press at the close of FY-71; *Carcinogenesis Abstracts* Volume VII has been published and distributed; accelerated work on Volume IX has brought the publication close to the desired currency level.

The Unit has assisted the Associate Scientific Director in developing a system of Carcinogenesis staff organization for effective management of the rapidly growing contract program.

Lung Cancer Unit: The program of the Lung Cancer Unit has been developed as an intramural focal point for the Special Lung Cancer Program. Its activity makes use of a broad spectrum of experimental techniques, all applied to the study of the pathogenesis of lung cancer and its inhibition. Current studies range from investigations on the carcinogenic effects of different chemicals on respiratory epithelium in the whole animal, to the definition of the short term effects of carcinogens on *in vitro* organ culture systems, to investigations of the molecular mechanism of action of such carcinogens in cell-free systems. The following is a summary of the problems which have been under investigation within the Lung Cancer Unit:

(1) *Animal studies of respiratory carcinogenesis (morphological and biochemical correlations)*-- The effects of various dosage schedules for intratracheal administration of polycyclic hydrocarbons, nitrosamines and/or other materials in induction of squamous cell carcinoma in respiratory epithelium of hamsters are being studied. The long-term animal studies are paralleled with studies of short-term effects. Some of the parameters being

measured are incidence and type of tumors, ultrastructural effects of carcinogens on respiratory epithelium, and biochemical changes induced in respiratory epithelium in response to the administration of the carcinogen. The last problem is being investigated in particular detail with respect to changes in RNA synthesis and processing which may be induced by agents such as benzpyrene. Methods have been developed for separating respiratory epithelium from underlying connective tissue and cartilage, and for preparing metabolically active subcellular particles, such as nuclei, from the epithelial cells.

(2) *Studies on the isolated tracheal epithelium of the hamster (short-term organ culture)* -- Methods have been developed for short-term organ culture of tracheas, during which time the epithelium may be exposed to any of a variety of radioisotopes. The epithelium is then scraped away from the rest of the trachea, and studies on the metabolism of the radioactive label can then be performed. Before the trachea is placed in the short-term organ culture, the donor animal may be treated with any of a variety of carcinogenic or anti-carcinogenic substances. This technique has been used particularly in conjunction with polyacrylamide-gel-electrophoretic and radioautographic studies of RNA metabolism, with the carcinogens studied being benzpyrene, nitrosomethylurea, and diethylnitrosamine. The anti-carcinogenic substance has been vitamin A. Water-soluble carcinogens, such as nitrosomethylurea and diethylnitrosamine can be applied directly to the isolated trachea and can be shown to produce ultrastructural lesions in short-term studies.

(3) *Studies on the cellular and biochemical mechanism of action of vitamin A* -- Since vitamin A is a required substance for normal differentiation of respiratory epithelium and since it blocks the actions of carcinogenic substances such as benzpyrene, both *in vivo* and *in vitro*, studies on the mechanism of action of this substance are being actively pursued. These studies include electron microscopic examination of respiratory epithelium, during both hypo- and hypervitaminosis A, as well as biochemical investigations on the role of vitamin A in controlling protein synthesis in normal and malignant epithelial cells.

(4) *Studies on the control of nuclear RNA metabolism in tracheal epithelial cells* -- Since abnormal nuclear and nucleolar morphology are frequently used criteria for diagnosis of the malignant state in respiratory epithelium, studies on the factors controlling synthesis and processing of nuclear RNA are being conducted. These studies are currently focusing on the role of the nucleolus in 2'-O-methylation of RNA, and the effect of respiratory carcinogens on this process. Within the Lung Cancer Unit, new biochemical methodology, including specialized techniques of gel electrophoresis and chromatography of RNA, has just been developed, which will allow extensive new molecular studies on the role of RNA methylation in respiratory carcinogenesis.

Serial No. NCI-4696
1. Office of the Associate Scientific Director, Carcinogenesis, Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of Carcinogenic Hazards

Previous Serial Number: None

Principal Investigator: Umberto Saffiotti, M.D.

Other Investigators: R. R. Bates, M. A. Schneiderman, E. K. Weisburger, J. H. Weisburger, J. A. Cooper, J. A. Peters, and H. R. Rosenberg

Cooperating Units: Collaboration from the Staff of the Carcinogenesis Area and outside advisors

Man Years:
Total: 1.5
Professional: 1.5
Other: 0.0

Project Description

Objectives:

To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic hazards by chemical and physical agents and for the prevention of their carcinogenic effects in man.

Methods Employed:

Scientific coordination of the intramural and collaborative programs of the Carcinogenesis Area, Etiology, NCI. Convening expert advisory groups. Consultation with other Government agencies. Review of scientific information.

Major Findings:

A major effort has been made to define the guidelines for the evaluation of carcinogenic hazards. An Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens was convened at the request of the Surgeon General, USPHS. Its report, entitled "Evaluation of Environmental

Carcinogens", transmitted to the Surgeon General in May 1970, has been edited and submitted for publication in the *J. Nat. Cancer Inst.*

The report proposed new approaches to the problem of carcinogenesis evaluation, with emphasis on the following points: (a) any substance shown conclusively to produce tumors in animals should be considered carcinogenic and therefore a potential cancer hazard for man; (b) no level of exposure to a chemical carcinogen should be considered toxicologically insignificant for man: a "safe level" cannot be established for carcinogenic agents, and the concept of "socially acceptable risk" represents a more realistic notion; (c) the principle of zero tolerance should be retained for regulatory action, and exceptions should be made only after the most extraordinary justification and documentation; (d) no substance should be allowed for wide-spread human intake without having been properly tested for carcinogenicity and found negative. Methods for dose-response evaluation have been proposed. A conceptual scheme for the information required for the evaluation of carcinogenic risks for man has been outlined: it identifies the need for chemical analytical inputs, for biological data inputs and for arbitrary selection inputs.

A review of the dose-response relationship in carcinogenesis has been prepared.

Numerous contributions have been made towards the establishment of national policies in this field through discussions and documentation provided to Congressional committees, Government offices, departments and agencies, and scientific societies.

Significance to Biomedical Research and the Program of the Institute:

The national policies on environmental health should be based on sound scientific grounds. The Carcinogenesis Area at NCI provides a central source of information and competence in this field.

Proposed Course of Project:

Continuation of these functions.

Honors and Awards

Chairman, Chemical Carcinogenesis Contract Review Committee, Etiology, NCI

Chairman, Carcinogenesis Intramural Program Group, Etiology, NCI

Chairman, Interim Collaborative Program Review Group, Etiology, NCI

Chairman, Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens. Office of the Deputy Assistant Secretary for Health and Scientific Affairs, DHEW

Member, Etiology Program Management Group, NCI

Member, Tobacco Working Group, Lung Cancer Task Force, NCI

Chairman, Bioassay Subgroup of the Tobacco Working Group, Lung Cancer Task Force, NCI

Member, Ad Hoc Advisory Committee for the Registry of Experimental Cancers, NCI

Alternate Member for NIH, Departmental Pesticide Coordinating Committee, DHEW

Member, Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer, WHO

Participant and Chairman of the Panel on Biology at the International Study Group on Tobacco and Health, organized by the American Health Foundation, New York, New York, October 1970

Lecturer, Washington University School of Medicine, Department of Pathology, St. Louis, Missouri, November 1970

Discussant of Session VI: Toxicology, Pulmonary and Systemic Pathology, and participant at the Symposium on Hamster Pathology, organized by Bio-Research Institute, Cambridge, Massachusetts, November 1970

Lecturer, M. Negri Institute of Pharmacological Research, Milan, Italy, December 1970

Participant and Chairman of Session IV: Mechanisms of Cancer Induction II at the Twenty-Fourth Annual Symposium on Fundamental Cancer Research, organized by the M. D. Anderson Hospital and Tumor Institute, Houston, Texas, March 1971

Speaker at the Symposium on Dose Response in the Appraisal of Toxicologic Effects at the Tenth Annual Meeting of the Society of Toxicology, Washington D.C., March 1971

Participant and Co-Chairman of Carcinogenesis IV: Drugs; Various Sites, at the Sixty-Second Annual Meeting of the American Association for Cancer Research, Chicago, Illinois, April 1971

Publications

Saffiotti, U.: Food additives in relation to environmental cancer in man. In Clark, R. L., Cumley, R. W., McCay, J., and Copeland, M. M. (Eds.): *Oncology 1970: A. Environmental Causes; B. Epidemiology and Demography; C. Cancer Education* (Proceedings of the Tenth International Cancer Congress 1970). Chicago, Illinois, Yearbook Medical Publishers, Inc. (In Press).

Saffiotti, U.: Mechanisms of cancer induction in relation to the problem of environmental cancer. In *Environment and Cancer* (Proceedings of the Twenty-Fourth Annual Symposium on Fundamental Cancer Research). Baltimore, Maryland, Williams & Wilkins (In Press).

Serial No. NCI-4697
1. Office of the Associate Scientific Director, Carcinogenesis, Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental Respiratory Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Umberto Saffiotti, M.D.

Other Investigators: R. Montesano, A. R. Sellakumar, and D. G. Kaufman

Cooperating Units: The International Agency for Research on Cancer, WHO, Lyon, France and The Eppley Institute for Research in Cancer, The University of Nebraska, Omaha, Nebraska

Man Years:
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description

Objectives:

To elucidate the causative factors and pathogenetic mechanisms of respiratory tract cancer and to define related biological models for the experimental study of bronchogenic carcinoma.

Methods Employed:

(1) Coordination of the Special Lung Cancer Program in the Carcinogenesis Area, Etiology, NCI. (2) Experimental studies, including some initiated by the Principal Investigator prior to his assumption of duties at NIH, have been completed. They include dose-response studies using Syrian golden hamsters with (a) benzo[*a*]pyrene administered intratracheally as a particulate suspension attached to particles of ferric oxide; and (b) diethylnitrosamine administered systemically to adult or newborn hamsters. Studies employing a combination of these two respiratory carcinogens were continued. (3) *In vitro* techniques and biochemical methods as reported in the individual project reports of the Lung Cancer Unit.

Major Findings:

(1) A Lung Cancer Unit has been established and staffed to provide an intramural laboratory focus for the development of the Special Lung Cancer Program. Two review symposia were organized jointly with the Atomic Energy Commission, on "Inhalation Carcinogenesis" and "Morphology of Experimental Respiratory Carcinogenesis". (2) Detailed histopathological study of the respiratory tumors induced by a variety of topical and systemic treatments as previously reported has shown a segmental response of the respiratory tract with specific differentiation patterns of tumors resulting from different types of treatments. A morphological analysis has been made and a classification of respiratory tumors proposed. Dose response studies show that the segmental response is found to follow the dose of carcinogen both in terms of site of tumor development and of morphological differentiation. (3) Methodologies have been developed for the study of morphological-biochemical correlations in respiratory carcinogenesis. They will provide a definition of the points of attack of carcinogens on their target sites in the respiratory epithelium, as a basis for inhibition. Also, these methods make it possible to study the problem of the relationship between epithelial cell differentiation and carcinogenesis, a crucial issue in the pathogenesis of bronchogenic carcinoma (see individual reports of Lung Cancer Unit).

Significance to Biomedical Research and the Program of the Institute:

These studies are an integral part of the effort aimed at defining the factors leading to the development of bronchogenic carcinoma in animal systems in conditions that closely resemble those of human lung cancer development. The methods developed in the course of this project are now receiving wide application in several laboratories for studies on the pathogenesis of lung cancer, and have been recommended as a basis for further development of bioassays for respiratory carcinogens, including tobacco smoke, by the Tobacco Working Group of the Lung Cancer Task Force.

Proposed Course of Project:

Continuation of studies closely related to the new Special Lung Cancer Program developed by the Carcinogenesis Area.

Publications

Saffiotti, U.: Morphology of respiratory tumors induced in Syrian golden hamsters. In Nettesheim, P., Hanna, M. G., Jr., and Deatherage, J. W., Jr. (Eds.): *Morphology of Experimental Respiratory Carcinogenesis*. Atomic Energy Commission Symposium Series, No. 21 (CONF-700501). Oak Ridge, Tennessee, U. S. Atomic Energy Commission, Division of Technical Information Extension, 1970, pp. 245-254.

Smith, J. M., Sporn, M. B., Berkowitz, D. M., Kakefuda, T., Callan, E., and Saffiotti, U.: Isolation of enzymatically active nuclei from epithelial cells of the trachea. *Cancer Res.* 31: 199-202, 1971.

Serial No. NCI-4697

Saffiotti, U., Dunn, T., Kuschner, M., Mohr, U., Nettesheim, P., Stanton, M., and Turusov, V.: Panel discussion: Recommendations for classification of lung tumors in animals. In Nettesheim, P., Hanna, M. G., Jr., and Deatherage, J. W., Jr. (Eds.): *Morphology of Experimental Respiratory Carcinogenesis.* Atomic Energy Commission Symposium Series, No. 21 (CONF-700501). Oak Ridge, Tennessee, U. S. Atomic Energy Commission, Division of Technical Information Extension, 1970, pp. 451-472.

Serial No. NCI-4695
1. Office of the Associate Scientific Director, Carcinogenesis, Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Coordination of Collaborative Research

Previous Serial Number: None

Principal Investigator: Allen H. Heim, Ph.D.

Other Investigators:

Cooperating Units: Carcinogenesis Staff

Man Years:
Total: 0.3
Professional: 0.3
Other: 0.0

Project Description

Objectives:

To determine and implement systematic methods for receiving, reviewing, initiating, and managing research proposals within the collaborative portion of the Chemical Carcinogenesis Program.

Methods Employed:

Standard science management methods which will be adapted to the dual review system and which will be designed to maximize the effects of a cooperative working relationship between the scientist and scientist-administrator.

Major Findings:

Formal and concentrated effort has just been initiated.

Significance to Biomedical Research and the Program of the Institute:

Effective management of the collaborative program, if conducted in the context of good science supported by good management, should increase the efficiency and effectiveness of the planning process, the staff efforts and the outcome in relation to overall program objectives and responsibilities.

Proposed Course of Project:

Implementation as soon as practicable.

Serial No. NCI-4799
1. Office of the Associate Scientific Director, Carcinogenesis, Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of Improved Standards for Intramural Animal Care

Previous Serial Number: None

Principal Investigator: Raymond F. Newell

Other Investigators: Carcinogenesis Staff

Cooperating Units: Engineering and Sanitation Section, Environmental Services Branch, DRS

Man Years:
Total: 0.9
Professional: 0.7
Other: 0.2

Project Description

Objectives:

To provide uniformity of animal care throughout the Carcinogenesis Area and Building 37, to achieve maximum utilization of existing animal facilities, equipment, and personnel and to increase the quality and effectiveness of research as it pertains to animal care.

Methods Employed:

Periodic inspection of current animal-holding facilities, meetings with staff to discuss problem areas, and development of guidelines for more efficient animal care.

Major Findings:

There is at present no centralized control over animal care in the intramural operations of Carcinogenesis. Each investigator has responsibility over the operation of his animal facility. As a result of this, there is a complete lack of coordination as to animal care; the quality varies markedly. Further, this lack of coordination makes the implementation of adequate operating and sanitary procedures impossible. Poor coordination also prevents improvement of the existing service facilities and eliminates the attainment of greater operating efficiency.

Significance to Biomedical Research and the Program of the Institute:

Without clean animal facilities and proper ventilation, the quality and validity of experiments is questionable. With well-designed animal facilities, as well as routine inspections and discussions, complex and extensive experiments may be expected to yield increasingly reliable results.

Proposed Course of Project:

With the assistance and cooperation of representatives from Etiology, Chemotherapy, General Laboratories and Clinics, and the Head of Building 37's general service group, an animal care panel will be instituted to implement adequate operating and sanitary procedures. The Panel would analyze the problem and make recommendations as to improvements for the attainment of greater operating efficiency.

Honors and Awards

Executive Secretary, Chemical Carcinogenesis Contract Review Committee, Etiology, NCI

Executive Secretary, Interim Collaborative Program Review Group, Etiology, NCI

Publications

Nettesheim, P., Hanna, M. G., Jr., Doherty, D. G., Newell, R. F., and Hellman, A.: Effects of chronic exposure to artificial smog and chromium oxide dust on the incidence of lung tumors in mice. In Hanna, M. G., Jr., Nettesheim, P., and Gilbert, J. R. (Eds.): *Inhalation Carcinogenesis*. Atomic Energy Commission Symposium Series, No. 18 (CONF-691001). Oak Ridge, Tennessee, U. S. Atomic Energy Commission, Division of Technical Information Extension, 1970, pp. 305-320.

Nettesheim, P., Hanna, M. G., Jr., Doherty, D. G., Newell, R. F., and Hellman, A.: Effect of chronic exposure to air pollutants on the respiratory tracts of mice: Histopathological findings. In Nettesheim, P., Hanna, M. G., Jr., and Deatherage, J. W., Jr. (Eds.): *Morphology of Experimental Respiratory Carcinogenesis*. Atomic Energy Commission Symposium Series, No. 21 (CONF-700501). Oak Ridge, Tennessee, U. S. Atomic Energy Commission, Division of Technical Information Extension, 1970, pp. 437-448.

Serial No. NCI-4763
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Nuclear Exoribonucleases from Normal and Tumor Cells

Previous Serial Number: Same

Principal Investigator: Michael B. Sporn, M.D.

Other Investigator: Mary S. Baker, M.S.

Cooperating Units: None

Man Years:
Total: 0.5
Professional: 0.3
Other: 0.2

Project Description

Objectives:

To characterize the enzymes of the normal and tumor cell nucleus which are responsible for the in situ destruction of the newly synthesized, nascent messenger RNA of the nucleus. To define the role of these enzymes in the cell nucleus, particularly with respect to control of gene action.

Methods Employed:

Highly purified nuclei have been isolated from many cell types, including tracheal epithelial cells, HeLa cells, and Ehrlich ascites tumor cells. The exoribonuclease which degrades messenger RNA in these nuclei has been highly purified. Inhibitors have been tested by classical enzymological methods for their ability to irreversibly inactivate exoribonuclease, without inactivating enzymes of RNA synthesis.

Major Findings:

Exoribonuclease has been identified for the first time in the nucleus of tracheal epithelial cells. Exoribonuclease has been shown to be irreversibly inhibited by agents such as 3'-chloroacetylamino-3'-deoxythymidine-5'-phosphate and thymidine-3'-p-carbethoxyamino-phenylphosphate, at concentrations at which RNA polymerase is unaffected. These studies further corroborate previous studies, which have shown that it is possible to irreversibly inhibit the nuclear enzyme which destroys messenger RNA without inhibiting the nuclear enzyme which synthesizes messenger RNA.

Significance to Biomedical Research and the Program of the Institute:

The role of the gene action system is a central problem in all studies of carcinogenesis, whether chemical or viral. Research on the factors which control gene transcription and translation is among the most basic in all of cancer research. Since messenger RNA is the primary product of gene transcription, it is of utmost importance to know the mechanisms which control both its synthesis and degradation. The present project is yielding data on the enzymes and mechanism of messenger RNA destruction in both normal and cancer cells.

Proposed Course of Project:

To continue studies on the molecular mechanisms whereby messenger RNA is destroyed in normal cells and cancer cells. To evaluate the role of enzymes of messenger RNA destruction in controlling the growth and differentiation of both normal and cancer cells, and in particular to focus on the effect of carcinogens on the activity of these enzymes.

Honors and Awards

M. B. Sporn, Invited to lecture, Duke University Medical Center, Durham, North Carolina, Department of Medicine, March, 1971.

M. B. Sporn, Invited to lecture, Washington Hospital Center, Washington, D.C., Department of Medicine, April, 1971

Publications

1. Glinski, R.P., Ash, A.B., Stevens, C.L., Sporn, M.B., and Lazarus, H.M.: Nucleotide synthesis. I. Derivatives of thymidine containing p-nitrophenyl phosphate groups. J. Org. Chem. 36: 245-249, 1971.
2. Lazarus, H.M. and Sporn, M.B.: Studies on the allosteric properties of nuclear exoribonuclease from Ehrlich ascites tumor. Biochemistry 10: 505-510, 1971.

Serial No. NCI-4777
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Carcinogens on Methylation of Nuclear RNA

Previous Serial Number: Same

Principal Investigators: Adhid Al-Arif, Ph.D. and Michael B. Sporn, M.D.

Other Investigators: Joseph M. Smith

Cooperating Units: None

Man Years:
Total: 1.3
Professional: 1.2
Other: 0.1

Project Description

Objectives:

The nucleolus is known to methylate RNA on the 2'-oxygen of ribose. Since nucleolar lesions are known to be a prominent feature of cells treated with chemical or viral carcinogens, it is important to evaluate whether carcinogens affect 2'-O-methylation of RNA. Failure to methylate RNA would lead to excessive destruction of RNA within the nucleus, and failure to transport RNA to the cytoplasm.

Methods Employed:

Methylation of RNA is being studied in two different systems: 1) the isolated hamster tracheal organ fragment, and 2) isolated rat liver nuclei. New chromatographic methods have been developed to separate methylated nucleosides from normal nucleosides. RNA on acrylamide gels can be quantitatively converted to nucleosides and the extent of methylation measured. A new system for assay of methylation of RNA *in vitro* by isolated nuclei has also been developed.

Major Findings:

Normal tracheal RNA has been found to be 2'-O-methylated to the extent of approximately 1% of the total nucleotide residues. The effects of carcinogens on this process in the isolated trachea are currently being evaluated. The *in vitro* studies with rat liver nuclei are the first definitive studies on the ability of the nucleus to 2'-O-methylate RNA *in vitro*. The effects of actinomycin D on this process are currently being evaluated.

Significance to Biomedical Research and the Program of the Institute:

The problem of stabilization of newly synthesized RNA is a fundamental problem in cell biology and carcinogenesis. The nucleolus is believed to be a key intracellular organelle for RNA stabilization, and 2'-O-methylation of RNA is believed to be a key molecular step to this process in the nucleolus. It is well known that both chemical and viral carcinogens affect nucleolar ultrastructure. The above studies are thus aimed at elucidating a potentially critical molecular lesion in carcinogenesis.

Proposed Course of Project:

To continue as outlined above, and particularly to focus on the effects of different carcinogens on methylation of RNA in the tracheobronchial epithelium.

Serial No. NCI-4792
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Histogenesis of Squamous Cell Carcinoma of the Hamster Respiratory Tract Caused by Benzo[a]pyrene-Ferric Oxide

Previous Serial Number: None

Principal Investigator: Curtis Harris, M.D.

Other Investigators: Joseph M. Smith, Michael B. Sporn, M.D., David G, Kaufman, M.D., Umberto Saffiotti, M.D.

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.4
Other: 0.2

Project Description

Objectives:

This is a new project. Previous investigations of the histogenesis of squamous cell carcinoma in man and animal models have shown that respiratory epithelium becomes hyperplastic, squamous metaplastic and finally neoplastic. These sequential changes in morphology may be better understood and defined by ultrastructural analysis.

Methods Employed:

The method of Saffiotti and co-workers is used to administer intratracheal instillations of benzo[a]pyrene carried on ferric oxide particles in hamsters. This treatment produces squamous metaplasia and squamous cell carcinoma in the respiratory epithelium of the Syrian golden hamster. Following single or miltiple intratracheal instillations of 1) benzo[a]pyrene-ferric oxide; 2) pyrene-ferric oxide; 3) ferric oxide; or 4) saline, hamsters are sacrificed serially up to the time of gross tumor development. Specific segments of the trachea and bronchi are processed for high resolution light and electron microscopic study. Untreated and sham-treated animals are also examined.

Major Findings:

The ultrastructure of the hamster tracheobronchial epithelium is described for the first time. Acute and subacute effects of the above agents have been studied to date. The acute cellular changes in the tracheobronchial epithelium, produced by intratracheal instillations of benzo[a]pyrene carried by ferric oxide particles, included focal replacement of the columnar cells with pleomorphic cells. These had the ultrastructural features of atypical squamous cells, i.e., cytoplasmic filaments, widened intercellular spaces, abnormal desmosomes, and increased number of lysosomal derivatives. Polylobulated nuclei and pleomorphic nucleoli were prominent findings after multiple instillations of benzo[a]pyrene-ferric oxide. Defects in the basement membrane were also found. Intratracheal instillations of ferric oxide alone or pyrene-ferric oxide produced basal cell hyperplasia, which was reversible. The atypical squamous cells induced by benzo[a]pyrene-ferric oxide had ultrastructural features similar to hyperplastic epithelial cells described in the bronchi of smoking dogs and neoplastic squamous cells described in human bronchogenic carcinoma.

Significance to Biomedical Research and the Program of the Institute:

The incidence of bronchogenic squamous cell carcinoma in man has shown a progressive increase during the past five decades. The pathogenesis of bronchogenic carcinoma is being studied in this animal model by several experimental approaches by members of the Lung Cancer Unit. This multifaceted approach allows meaningful correlation of morphological and biochemical changes occurring during carcinogenesis. Our intramural investigations are closely coordinated with the lung cancer contract program.

Proposed Course of Project:

Continuation of the long-term histogenesis studies outlined above.

Serial No. NCI-4793
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1970 through June 30, 1971

Project Title: Development of Methods for *In Vitro* Metabolic Studies in Isolated Hamster Respiratory Tract Tissues

Previous Serial Number: None

Principal Investigators: David G. Kaufman, M.D., Mary S. Baker, M.S.

Other Investigators: Curtis C. Harris, M.D., Joseph M. Smith, and Michael B. Sporn, M.D.

Cooperating Unit: C. W. Dingman, M.D., Nucleic Acids Section, Chemistry Branch, NCI

Man Years:
Total: 1.3
Professional: 0.5
Other: 0.8

Project Description

Objectives:

The aim of this project is to provide a means of examining metabolic processes in a respiratory epithelium. The methods are designed to examine how metabolic processes are affected by chemical carcinogens administered either *in vivo* or *in vitro*.

Methods Employed:

Techniques have been developed for maintaining isolated hamster tracheas *in vitro* as organ cultures for at least four hours. Radioactively labeled precursors for protein and nucleic acid synthesis were added to the *in vitro* incubation medium and were incorporated into the organ fragment. The respiratory epithelium was scraped from the supportive structures of the trachea. Methods of preparing nucleic acid hydrolysates, acid insoluble residues, and quantitating DNA in epithelial scrapings have been devised. In addition, microchemical techniques were developed for isolation of undegraded RNA from the tracheal scrapings. These RNA preparations were examined by acrylamide gel electrophoresis. The same methods were employed where chemical carcinogens had been added during *in vitro* incubation, or where the animals from which tracheas were taken were pretreated with carcinogens *in vivo*.

Major Findings:

The respiratory epithelium of tracheas maintained in vitro in organ culture remained morphologically normal by light and electron microscopy through four hour incubations. Incorporation of tritiated leucine and uridine from the medium proceeded nearly linearly during three hour incubation periods. RNA was isolated from normal tracheas after incubation intervals of increasing length in the presence of a labeled RNA precursor. A pattern of isotopic incorporation into tracheal RNA fractions was essentially identical to that seen in other normal tissues. A similar experiment was performed using animals treated shortly (2 days) after an in vivo intratracheal instillation of the chemical carcinogen, benzo[a]pyrene, with hematite. The pattern of in vitro incorporation of isotope under these conditions was altered to a state which, in these preliminary studies, is not inconsistent with inhibited "maturation" of ribosomal RNA species.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program, designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits in vitro identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced in vivo. The methods developed permit examination of certain aspects of nuclear and cytoplasmic metabolism in viable respiratory epithelial cells. The effects of carcinogens administered in vitro can be examined biochemically. Furthermore, these methods will permit direct biochemical study of a respiratory epithelium during the course of in vivo carcinogenesis. This work coordinates with the in vivo carcinogenesis bioassays being conducted under contracts.

Proposed Course of the Project:

The emphasis of this project during the past year has been to develop methods for biochemical examination of respiratory tissue. Studies have concentrated on the normal epithelium. During the next year it is proposed to employ these methods for the examination of biochemical parameters of isolated tracheas during in vitro carcinogen administration and during in vivo carcinogenesis protocols.

Serial No. NCI-4794
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Autoradiographic Analysis of In Vitro Metabolism in Isolated Hamster Respiratory Tract Tissues

Previous Serial Number: None

Principal Investigators: David G. Kaufman, M.D., Curtis C. Harris, M.D., Joseph M. Smith

Other Investigators: Mary S. Baker, M.S., Michael B. Sporn, M.D., and Frank E. Jackson

Cooperating Unit: Dr. Hollis Boren, Marquette School of Medicine, Milwaukee, Wisconsin

Man Years:
Total: 1.3
Professional: 0.8
Other: 0.5

Project Description

Objectives:

The respiratory epithelium of the trachea is composed of a mixed cellular population. It is the aim of this new project to distinguish the metabolic activities of the various cell types and thus correlate biochemical and morphologic studies in the course of respiratory carcinogenesis. Changes in cellular populations, as well as changes in metabolic activities following carcinogen administration, are examined concurrently.

Methods Employed:

Radioisotopes are incorporated into isolated hamster tracheas in vitro using methods developed in this laboratory. Following in vitro incubation with isotope, autoradiograms are prepared from semi-thin and ultra-thin sections for light and electron microscopy. Grain counts are then performed on the developed autoradiograms, which distinguish the different cell types and location of grains over nuclei or cytoplasm. The collaboration of Dr. Boren's group is involved in the experimental design, grain counting, statistical analysis and interpretation of results.

Major Findings:

The cellular populations of tracheal epithelium maintained *in vitro* in organ culture incorporate radioactive tracers from the culture fluid at different rates. Preliminary observations suggest that mucus-secreting cells incorporate labeled leucine and uridine at a slightly greater rate than do ciliated cells; basal cells are far less active than either mucus-secreting or ciliated cells. Preliminary observation of the distribution of grains over nucleus and cytoplasm as a function of time of incorporation of labeled uridine, shows a correlation between the appearance of grains over the cytoplasm and the chemical detection of labeled mature ribosomal RNA species in RNA isolated from tracheal epithelium.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits *in vitro* identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced *in vivo*. The methods which have been developed permit some correlation of biochemical observations with respect to constituent cellular populations of this target tissue. Such correlations should be of critical interest where biochemical changes reflect changes in cellular population, such as those known to occur curing *in vivo* carcinogenesis.

Proposed Course of Project:

The emphasis of this project during the past year has been to develop methods and employ them to examine mostly normal respiratory epithelia. Future experiments will examine epithelia from animals exposed to carcinogens *in vivo* and *in vitro*. We also propose to examine the incorporation of radioactively labeled respiratory carcinogens when administered *in vitro*.

Serial No. NCI-4795
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical-morphological Studies on Hamster Respiratory Tract Epithelium: RNA Metabolism

Previous Serial Number: NCI-4775

Principal Investigator: Michael B. Sporn, M.D.

Other Investigators: Mary S. Baker, M.S., Joseph M. Smith, and Umberto Saffiotti, M.D.

Cooperating Units: None

Man Years:
Total: 0.8
Professional: 0.3
Other: 0.5

Project Description

Objectives:

The objective of this project is to develop new techniques and methods to study biochemical and morphological events that characterize the carcinogenic response of the respiratory epithelium. RNA metabolism is studied in hamster tracheal epithelium in normal conditions and under the effects of different vitamin A levels and with chemical carcinogens which cause cancer of the respiratory tract.

Methods Employed:

Techniques have been developed to scrape the tracheal epithelium free from the rest of the trachea. Micro detergent methods have been developed to prepare pure nuclear and cytoplasmic fractions from these cells. Electron microscopy has been used to monitor cell fractionation. Microchemical techniques have been developed to measure amounts of RNA and DNA in epithelial samples. Hamsters have been raised on vitamin A deficient diets, and chemical measurement of vitamin A in their livers shows no detectable vitamin A after four weeks on the deficient diet.

Major Findings:

Isolated, clean nuclei can be prepared from tracheal epithelium in 50% yield. RNA polymerase activity can be assayed in these nuclei under high salt or low salt conditions. Chemical carcinogens, such as benzo[*a*]pyrene, when administered intratracheally 48 hours before sacrifice, cause a two fold increase in RNA polymerase activity. Benzpyrene also markedly changes the morphology of the nucleoli in the isolated nucleoli. The effects of vitamin A on RNA metabolism in the nucleus are currently under evaluation.

Significance to Biomedical Research and the Program of the Institute:

Respiratory carcinogenesis is a fundamental area of interest in the program of the Institute. This project contributes to the lung cancer program with the development of biologic models for the study of respiratory carcinogenesis. Tracheobronchial epithelium is the target tissue for the most common respiratory carcinogens. The effects of these carcinogens on nucleic acid metabolism in the target tissue can now be studied with a variety of *in vivo* and *in vitro* techniques. Benzpyrene has been found to have a marked effect on nuclear RNA metabolism. Moreover, the possible effects of vitamin A (which is both a differentiation hormone and a modifier of carcinogenesis for this particular tissue) on nucleic acid metabolism in respiratory epithelium can now be studied by a variety of biochemical methods.

Proposed Course of Project:

To continue studies as outlined above.

Publications

Smith, J.M., Sporn, M.B., Berkowitz, D.M., Kakefuda, T., Callan, E., and Saffiotti, U.: Isolation of enzymatically active nuclei from epithelial cells of the trachea. *Cancer Research* 31: 199-202, 1971.

Serial No. NCI-4796
1. Office of the Associate Scientific Director for Carcinogenesis
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Squamous Metaplasia of the Hamster Respiratory Epithelium Induced by Vitamin A Deficiency or Benzo[a]pyrene-Ferric Oxide

Previous Serial Number: None

Principal Investigator: Curtis C. Harris, M.D. and Joseph M. Smith

Other Investigators: Michael B. Sporn, M.D., David G. Kaufman, M.D., Frank E. Jackson, and Umberto Saffiotti, M.D.

Cooperating Units: Histopathology Unit, Experimental Pathology Branch, NCI

Man Years:
Total: 0.5
Professional: 0.4
Other: 0.1

Project Description

Objectives:

Mucous and ciliated cells of the respiratory epithelium provide a defense against inhaled pollutants and carcinogens. This columnar epithelium can undergo squamous metaplasia, which may be a morphological stage in the histogenesis of squamous cell carcinoma. It is of interest to compare squamous metaplasia induced by carcinogens to squamous metaplasia found during vitamin A deficiency.

Methods Employed:

Hamsters were maintained on a vitamin A-deficient diet. Animals were examined during 4 stages of deficiency based upon their weight course and clinical signs. Pair-fed animals served as controls. A second group of animals received 10 intratracheal instillations of benzo[a]pyrene-ferric oxide. Specific tracheal and bronchial rings were processed for high resolution light and electron microscopic study.

Major Findings:

The sequential changes in the trachea during vitamin A deficiency appear to be: 1) the ciliated and mucous cells flatten; 2) basal cells increase in number; 3) a widened intercellular space develops between the hyperplastic layer of basal cells and the luminal cells are sloughed; 4) basal cells differentiate into flat layers of squamous cells. As described previously by us, intratracheal instillations of benzo[a]pyrene-ferric oxide cause many histological changes including squamous metaplasia with atypical squamous cells. Benzo[a]pyrene-ferric oxide also causes a regular squamous metaplasia which could not be distinguished from squamous metaplasia induced by vitamin A at the light microscopic level, whereas abnormal nucleoli and defects in the basement membrane were apparent at the ultrastructural level. Defects in the basement membrane have been observed in preneoplastic and neoplastic lesions of the skin, mammary gland and larynx.

Significance to Biomedical Research and the Program of the Institute:

Vitamin A has a pivotal role in the control of epithelial differentiation in the respiratory epithelium. The differentiation of basal cells into mucous and ciliated cells is dependent on adequate levels of vitamin A. Vitamin A deficiency results in an alternate path of differentiation, *i.e.*, squamous metaplasia, which also appears to be a preneoplastic stage in the histogenesis of squamous cell carcinoma of the bronchi. Hypervitaminosis A has been found to decrease the incidence of squamous metaplasia and squamous carcinoma in this animal model. Squamous metaplasia induced by benzo[a]pyrene-ferric oxide can be distinguished at the ultrastructural level from squamous metaplasia observed during vitamin A deficiency. This study is designed to contribute to the elucidation of the control mechanisms involved in epithelial differentiation and carcinogenesis.

Proposed Course of Project:

Completion of the above experiments. Determine the effect of hypervitaminosis A on squamous metaplasia caused by benzo[a]pyrene-ferric oxide.

Serial No. NCI-4798
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Control of Epithelial Cell Differentiation and Carcinogenesis

Previous Serial Number: None

Principal Investigator: Luigi M. De Luca, Ph.D.

Other Investigators: Nancy E. Maestri, Curtis C. Harris, M.D., David G. Kaufman, M.D., Adhid Al-Arif, Ph.D., Michael B. Sporn, M.D., Mary S. Baker, M.S., Joseph M. Smith, and Umberto Saffiotti, M.D.

Cooperating Units: None

Man Years:
Total: 0.8
Professional: 0.4
Other: 0.4

Project Description

Objectives:

To study the control of mucus-squamous cell differentiation by vitamin A in the respiratory tract and in other mucus-secreting and keratinizing tissues.

Methods Employed:

The study of translational and post-translational control of cell differentiation by retinol will be continued. The study of the biosynthesis of specific glycopeptides by using specific precursors will be carried out in whole cell and cell-free systems from tracheal and intestinal epithelia. Changes in goblet cell formation or disappearance in response to vitamin A or carcinogen treatments will be followed by a specific immunofluorescence assay recently developed by the principal investigator at the Massachusetts Institute of Technology.

Major Findings:

The observation was made that vitamin A deficiency leads to a decrease in the number of goblet cells and in the incorporation of C^{14}-glucosomine into a specific glycopeptide. This glycopeptide was shown to be present in small intestinal mucosa and respiratory tract of the hamster. By using the indirect immunofluorescent technique, we were able to locate the glycopeptide uniquely in goblet cells. This easy assay can provide an efficient and rapid tool to study mucus cell formation in response to factors influencing the differentiation of mucus-secreting epithelia, such as respiratory carcinogens and different levels of retinol.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the identification of key control mechanisms of carcinogenesis: it is a part of the Lung Cancer program, addressed to the development of mechanisms of inhibition and control of respiratory carcinogenesis. The control of mucus cell differentiation by vitamin A is also of great interest in helping to understand the mechanism of carcinogenesis in epithelial tissues in general. The vitamin allows formation of goblet cells and its absence leads to hyperplasia of basal cells, squamous metaplasia and keratinization: a sequence of events similar to what takes place in the respiratory epithelia after exposure to benzopyrene.

The fucose-glycopeptide, shown to be present only in goblet cells and to depend on vitamin A for its synthesis, can be used as a marker for differentiative responses in epithelia. The immunofluorescence assay will be of help in these studies.

Proposed Course of Project:

To continue studies as outlined above.

Serial No. NCI-4797
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Acute and Chronic Effects of Carcinogenic N-nitroso Compounds in the Respiratory Epithelium

Previous Serial Number: None

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: Michael B. Sporn, M.D., David G. Kaufman, M.D., Joseph M. Smith, Frank E. Jackson, Umberto Saffiotti, M.D.

Cooperating Units: Histopathology Unit, Experimental Pathology Branch, NCI

Man Years:
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description

Objectives:

N-nitroso compounds, such as diethylnitrosamine (DEN), dimethylnitrosamine (DMN) and N-methyl-N-nitrosourea (NMH) are respiratory carcinogens. NMH is a proximate carcinogen, whereas DMN and DEN require metabolism into active form(s). Intratracheal instillations of NMH induces squamous cell carcinoma of the lung as shown by Herrold. The objectives of this project are: 1) to study the histogenesis of squamous cell carcinoma caused by multiple instillations of NMH and compare these changes with those from a similar histogenesis study using a polynuclear hydrocarbon, benzo[a]pyrene; and 2) relate the acute ultrastructural lesions induced by these compounds to changes in RNA metabolism and carcinogenesis.

Methods Employed:

DEN, DMN and NMH are given by parenteral route or by intratracheal instillation. At selected times, animals are sacrificed and tracheal rings are processed for light and electron microscopic examination. The effect of these carcinogens on the incorporation of uridine-H^3 into cellular RNA is being studied by autoradiography, which allows the identification of the various cell populations in the tracheal epithelium. Multiple instillations of NMH are being given to hamsters for a serial sacrifice study of tumor histogenesis.

Major Findings:

This is a new project. Preliminary evidence indicated that DEN, DMN and NMH all cause acute alterations in nucleolar ultrastructure. Autoradiographic studies concerning the effects of these agents on RNA metabolism and the chronic effects of NMH have been initiated.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. N-nitroso compounds are a group of systemic carcinogens, which have marked tissue specificity. Their role in respiratory carcinogenesis is being actively investigated in several projects of the lung cancer program. The morphological and biochemical effects of these agents directly on respiratory epithelia are being compared to those of the carcinogenic polynuclear hydrocarbons.

Proposed Course of Project:

Continuation of these recently initiated studies.

Serial No. NCI-4699
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Carcinogenesis Bioassay Data System

Previous Serial Number: Same

Principal Investigators: Norbert P. Page, D.V.M.; John A. Cooper, II, Ph.D.; Howard R. Rosenberg, M.S.; and Umberto Saffiotti, M.D.

Other Investigators: Mary S. Linhart

Cooperating Units: Data Management Branch, Division of Computer Research and Technology, NIH

Man years:
Total: 0.95
Professional: 0.75
Other: 0.20

Project Description

Objectives:

To develop a comprehensive data system for collection, storage, manipulation and recall of selected information which characterizes carcinogenesis bioassay studies. In doing so, to create and maintain a file of data about substances studied as part of the Chemical Carcinogenesis Program.

Methods Employed:

Standard systems design methods and automated data processing techniques are utilized in full context of the NIH/DCRT hardware and software environment.

Major Findings:

During the past year, the Carcinogenesis Bioassay Data System (CBDS) was programmed according to specifications developed through prior systems analysis. A test of the system and its input forms was made in cooperation with a current bioassay contractor. In response to the experience, modifications of programs and input forms were made to refine the CBDS and prepare it for pilot testing by several contractors. The seven types of input forms correspond respectively to the following experimental components about which data are collected: Chemical Batch, Treatment Preparation, Animal Colony, Animal Group, Experimental Environment, Cage Observation, and Individual Animal. A chemical data subsystem (former Project Serial No. NCI-4790) has been merged into the CBDS. The former contains the following data on substances under investigation:

1. Name (Chemical Abstracts preferred, inverted form)
2. Synonyms (common and trade names)
3. Wiswesser Line Notation
4. Production data
5. Manufacturers
6. Fragment codes
7. Chemical Abstracts Registry Number
8. Molecular formula

Significance to Biomedical Research and the Program of the Institute:

Because of the size, diversity and complexity of the Carcinogenesis Bioassay Program, it is essential that a method be available to manage and make optional use of the information generated by its various activities. The Carcinogenesis Bioassay Data System will provide this and will, in addition, contribute to uniformity of reporting results of bioassay studies and to standardization of chemical and tumor nomenclature. It will also provide for routine rapid evaluation of bioassay studies, including comparison of results from multiple sources, and for ready access to summary data for statistical analysis.

Proposed Course of Project:

Schedule for implementation of the operational phase of the Carcinogenesis Bioassay Data System is as follows:

April 1971 - Receipt of revised input forms and initiation of pilot use.
September 1971 - Total implementation.
December 1971 - Extension of system to include sophisticated data analysis.

Serial No. NCI-4791
1. Office of the Associate Scientific Director for Carcinogenesis, Etiology
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Carcinogenesis Contract Data System

Previous Serial Number: Same

Principal Investigator: Howard R. Rosenberg, M.S.

Other Investigator:

Cooperating Units: None

Man Years:
Total: 0.1
Professional: 0.1
Other: 0.0

Project Description

Objectives:

To maintain a computerized data file to aid in the administration of the Chemical Carcinogenesis Contract Program.

Methods Employed:

Individual contract records contain information concerning contract identification, fields of contract work emphasis, fiscal actions, and monitoring activities. The file is updated monthly from an IBM/2741 terminal using the interactive program WYLBUR as supported by the NIH Division of Computer Research and Technology.

Major Findings:

Standard outputs from the system include the following:

1. Summary information reports on individual contracts.

2. Tables showing breakdown of Carcinogenesis and Lung Cancer contract expenditures by program segment.

3. Graphic representation of active time periods and associated fund allocations for specified contracts or groups of contracts.

Data is searched and manipulated to form special reports and responses to inquiries through simple sets of commands from the terminal.

Significance to Biomedical Research and the Program of the Institute:

Effective administration of the Carcinogenesis Contract Program requires data that are accurate, timely, and comparable. The Carcinogenesis Contract Data System provides such information to those concerned with overall program management as well as to individual project officers.

Proposed Course of Project:

Content of the system file will be reevaluated with respect to the benefits and costs of maintaining each piece of data. It is expected that minor changes in record structure will come of this examination. More extensive use and distribution will be made of the system reports as a segment oriented Carcinogenesis Contract Program management structure comes formally into being.

Summary Report
BIOLOGY BRANCH, ETIOLOGY
July 1, 1970 - June 30, 1971

Members of the Biology Branch continue to study host response to chemical carcinogenesis. These studies have led to the development of a short-term method that may be useful for the bioassay of chemical carcinogens and have contributed to an increased understanding of the immunologic reactivity related to the carcinogenic process. The contract program has been expanded so that recent advances in our knowledge of host responses to chemical carcinogenesis can be applied to problems in cancer etiology and control.

Highlights of Current Achievements in the Biology Branch

1. Tumor specific antigens have been extracted from tumor cells. The extracted antigens appear to be soluble in aqueous media; partial purification of these antigens has been achieved by standard methods of protein fractionation.

2. Each of 15 chemical carcinogens has been found capable of transforming hamster embryo cells *in vitro*. The transformed cells produced by carcinogenic polycyclic hydrocarbons are capable of inducing the formation of tumors in hamsters; none of the transformed cells produced by compounds of other classes has been tested as yet for neoplastic potential. Non-carcinogenic compounds failed to produce *in vitro* transformation.

3. Guinea pigs with established intradermal tumors and regional lymph node metastases can be cured by the injection of living *Mycobacterium bovis* (strain BCG) directly into the intradermal tumor.

4. About two weeks after beginning chronic administration to guinea pigs of a non-toxic, carcinogenic dose of diethylnitrosamine, the serum content of the fourth component of complement (C4) is reduced to less than 5% of the normal level; none of the other eight components of complement is significantly affected.

Molecular Separations Unit. This Unit continues to isolate and characterize biologically active substances that may be useful in the solution of problems in the etiology and control of cancer. Because of the need for criteria of *in vitro* transformation other than morphologic alteration (see report of Cytogenetics and Cytology Section), members of the Molecular Separations Unit are establishing methods for the solubilization of tumor specific antigens. The physicochemical and immunochemical properties of the soluble antigens are being studied to provide methods that can be used to determine how soon transformation occurs after addition of carcinogen to the cells. A method that appears to have applicability to the extraction of surface antigens from a wide variety of cells, has been used to solubilize tumor specific antigens from transplantable, diethylnitrosamine-induced, guinea pig tumors. Solubilization of the tumor antigens is achieved by suspending freeze-dried tumor cells in a hypertonic salt solution. The supernatant fluid, but not the insoluble cell debris, after concentration of the fluid and reduction of the salt concentration to isotonicity is capable of eliciting in the skin of immunized guinea pigs a delayed hypersensitivity reaction indistinguishable from that induced by the injection of living tumor cells. As judged by the degree of skin reactivity the yield of soluble antigen is a significant proportion of that found in the living tumor cells. Further purification of the solubilized tumor antigen has been achieved by ammonium sulfate precipitation and gel filtration, with excellent recovery of active material at each step. These immunochemical methods will be applied to the study of transformed guinea pig embryo cells and to the study of transformed human embryo cells.

An important contribution to histopathology has been the demonstration that complement components are bound *in vivo* to tissues and organs associated with a variety of immunopathologic processes. Accordingly, members of the Molecular Separations Unit and the Immunochemistry Section have devised a new, extremely sensitive and accurate method for the detection of C3 (the third component of complement) bound to cell surfaces. This method will be applied to studies with tumor-bearing guinea pigs to determine the role of circulating antibodies in the carcinogenic process.

Cytogenetics and Cytology Section. The Cytogenetics and Cytology Section continues to carry out *in vitro* and *in vivo* experiments designed to increase our understanding of the processes of chemical carcinogenesis. Emphasis has been placed on the development of reliable short-term methods for the bioassay of chemical carcinogens and on the study of the effects of these compounds on cell genetics. Carcinogens and related non-carcinogenic compounds are studied in terms of both morphological and biochemical changes in cells; also studied are the effects of inhibitors and promoters of neoplastic transformation. The Section utilizes a variety of models which include cell culture, bacteriophage induction, reversion to prototrophy of bacteria, and newborn, pregnant, and weanling animals.

Results of these studies have provided information about the effects of chemical carcinogens on susceptible and non-susceptible cells and about host responses to cellular changes induced by chemical carcinogens. Transformation *in vitro* was defined as a criss-crossing of cells that produced altered colonies not seen in controls.

The morphology, neoplastic potential and chromosomal characteristics of the cell lines derived from colonies isolated after chemical exposure were correlated. The patterns of various cell lines when produced by the same carcinogen differed in terms of types of cells present as well as degree of altered growth pattern. Cloning experiments resulted in the formation of colonies which usually had more than one type of morphological transformation. Cells of established lines derived from each culture transformed by a polycyclic hydrocarbon resulted in tumor formation when tested by subcutaneous inoculation into hamsters. No tumors were produced when control cells were inoculated into animals. Characteristic of the transformed cell lines analysed for karyological changes was the low incidence of chromosomal markers. The cell lines, regardless of carcinogen used for transformation, had chromosome modes which were classified as hypodiploid or hyperdiploid; the tumor derived cultures were quite similar to the transformed cell line in chromosome mode except for two which had shifted to the hypotetraploid range. The lack of common karyotypic changes demonstrates that the alterations were trivial and represent a random occurrence which is independent of the transformation. Since the transformed colonies were obtained only with carcinogens and were responsible for cell lines which produced tumors when injected into hamsters whereas control untransformed cell lines failed to produce tumors, it is concluded that carcinogenesis can be studied by an *in vitro* model with a high degree of reliability and that *in vitro* studies are relevant to *in vivo* neoplasia.

The assay procedure based on *in vitro* transformation is now being adapted to test additional polycyclic hydrocarbons as well as other classes of chemical carcinogens. The hepatocarcinogen, 2-acetylaminofluorene (AAF) and its metabolites, N-hydroxy-AAF and acetoxy-AAF, were toxic; rare transformation was found with N-hydroxy-AAF and significant numbers of transformations were found with acetoxy-AAF. Two derivatives of dibenzanthracene, 5,6-dihydrodibenz(ah)anthracene and 5,6-dihydrodibenz(aj)anthracene, tested *in vitro* reflected their known *in vivo* carcinogenic potency. The carcinogens aflatoxin B_1, 11-methylcyclopenta(a)phenathen-17-one and 3-hydroxybenzo(a)pyrene were also active. $7\text{-OH-CH}_3\text{-12CH}_3\text{BA}$ and $12\text{-OH-CH}_3\text{-7-CH}_3\text{BA}$, derivatives of 7,12-dimethylbenz(a)anthracene, were weak. Single applications of either urethan, N-hydroxyurethan or diethyinitrosamine (DENA) were negative. However, one injection of DENA to pregnant hamsters 48 hours prior to killing produced transformed colonies when fetal cells were plated from a secondary culture. Thus, it is possible to assay compounds that require metabolic activation by first exposing the cells *in vivo*. Currently we are seeking stimulators and inhibitors of *in vitro* transformation.

In the past we have investigated a number of factors that can affect the frequency of *in vitro* transformation of hamster cells. Radiation has been implicated in carcinogenesis in man and animals; therefore, it has been used in our system. Irradiation of hamster cells prior to exposure to chemical carcinogen resulted in an enhancement of transformation dependent upon radiation and chemical doses and the interval between the two treatments. At this time we are attempting to determine whether irradiation increases the sensitivity of cells to chemical carcinogens or selects cells more likely to be transformed by chemical.

The *in vitro* Carcinogenesis Program also includes a contract involving definition of standard experimental conditions for a reproducible bioassay and a second contract (under negotiation) on the nature of interaction between chemical and viral carcinogenesis. The ongoing studies use established cell lines which are non-tumorigenic and fresh explants of human, guinea pig or hamster embryo material. These studies have resulted in the establishment of useful cell lines for bioassay. The use of enhancing agents with carcinogens is incorporated so as to provide a broad range for potentially carcinogenic agents. Such studies will be designed so as to elucidate the role of chemical carcinogens in promoting viral tumorigenesis and the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis.

The major aims of the proposed study will be as follows: 1) to determine dose-response curves for various chemical carcinogens which together with oncogenic and non-oncogenic viruses enhance tumorigenesis, and 2) to compare by karyological, serological and cytological methods the independent and combined effects of chemicals and viruses on cells during and after transformation.

Several compounds have been studied for their teratogenic effects. We report the effect of treatment of fertilized ova prior to implantation. N-hydroxyurethan and actinomycin D both known teratogens and carcinogens were used with cultivated 2 and 8 cell ova. After cultivation with different concentrations of chemicals the ova were washed repeatedly and reimplanted into other females which had been superovulated. These females were genetically different so that progeny developing from transplanted ova could be differentiated. The progeny which had been exposed as fertilized ova to chemical carcinogens did not have a significant incidence of tumors or malformations.

In another series of experiments hamster embryos were injected intra-amniotically (ia) with 6-aminonicotinamide (6-AN) on gestation day 7, 8 or 9 and examined near term. Untreated and control treated embryos had low rates of resorption and malformation. 6-AN injected ia produced offspring with multiple defects including exencephaly, cleft palate, anophthalmia, micrognathia, and hydrocephalus. In contrast to day-8 and -9 embryos, which responded to 5-μg doses of 6-AN, day-7 embryos required 50-100 μg before exhibiting abnormalities.

Previously we demonstrated that carcinogenic polycyclic hydrocarbons kill embryos but are not teratogenic to hamster fetuses (1968). When pregnant hamsters were pretreated with low doses of benzo(a)pyrene or phenobarbital there was a sharp decrease in embryolethality due to a large dose of carcinogen. From embryological data we concluded that in addition to maternal protection there is a possibility that the placentas are also involved in detoxification. With this technique it was not possible to demonstrate that these compounds are teratogenic for the golden hamster.

Cellular Immunity Section. The program of this Section is designed to provide a basis for the application of immunologic principles to studies of the carcinogenic process. It is assumed that carcinogenesis is a series of molecular and cellular events culminating in the appearance of palpable tumors. The ability to apply immunology to the study of each step leading to tumor formation depends on the characterization of tumor specific antigens and the mediators of immunologic reactivity elaborated by the host in response to these antigens. Current knowledge indicates that cellular mediators of tumor specific immunity are of greater benefit to the host than humoral (circulating antibodies) mediators. Indeed, there is some evidence that the importance of tumor specific antibodies may be in their ability to promote rather than inhibit the growth of tumors. Another factor to be considered in attempting to apply immunology to studies of chemical carcinogenesis is the choice of methods used to identify tumor specific antigenic reactivity. Several in vitro methods for measuring tumor specific reactivity are available but only those that rely on specific tumor cell killing can be trusted to yield information relevant to the end-point of carcinogenesis i.e., the appearance of tumors. If a method for detecting tumor antigens is used that is not based on tumor cell killing there is a risk that the information derived from the use of such methods will not be relevant to carcinogenesis at any step of the process. Accordingly, we have concentrated our efforts on the development of cell-mediated methods that have as their end-point the suppression of tumor growth in vivo. Other tests such as delayed cutaneous hypersensitivity, colony inhibition and inhibition of macrophage migration must show strict correlation with suppression of tumor growth before they can be used with complete confidence in studies designed to elucidate the process of carcinogenesis. So far, only in vivo tumor suppression is capable of providing the kind of information needed for our purposes. Tumor suppression in vivo can be tested either after immunization of a tumor-free animal (prevention model) or attempts can be made to reverse an established tumor by immunologic means (immunotherapy model). The most stringent test for the immunogenicity of a tumor specific antigen is based on the immunotherapy model. For this reason, a major portion of our effort has been devoted to the development of immunotherapy models.

In a previous annual summary we reported that development of an immunotherapy model with a transplantable guinea pig tumor that when injected intradermally grows for a while and then regresses. The same tumor injected intramuscularly or intraperitoneally induces fatal, metastatic disease. Recently we have been working with a transplantable guinea pig tumor that grows progressively no matter where it is injected and six days after injection into the skin produces regional lymph node metastases. We were unable by conventional means to demonstrate the antigenicity of this tumor. Our previous work on the demonstration that tumor growth is suppressed at sites of delayed cutaneous hypersensitivity (DCH) as well as Edmund Klein's successful treatment of human Basal Cell Carcinoma by the induction of DCH at the tumor site led us to a search for antigens capable of inducing the highest degree of DCH. Among various agents tested, living Mycobacterium bovis (strain BCG) were found to have the greatest capacity to induce DCH and to suppress tumor growth. Injection of living BCG directly into the intradermal established tumor caused regression of the tumor and there was no evidence of regional lymph node metastases. Established tumors in mice also regress when living BCG are injected into the tumor. There is no local growth of tumor when mixtures consisting of living tumor cells and living BCG are injected into the skin of normal guinea pigs. Injection of such mixtures produces a state of systemic immunity to the tumor. This vaccine is effective therapy for an animal with a relatively small tumor burden (10^5 to 10^6 tumor cells in an intradermal papule).

Optimal conditions for suppression of established tumors by the induction of delayed cutaneous hypersensitivity at the tumor site are being investigated. Present evidence indicates:

1. The tumor-bearing host must be capable of developing and expressing delayed cutaneous hypersensitivity.

2. Living BCG provide the greatest reactivity of all allergens tested so far.

3. Best results are obtained by injecting BCG directly into the tumor.

4. The effective dose range of BCG begins at 10^6 organisms per injection.

5. Tumors up to 0.5 gm (1 cm in diameter) can be cured by direct injection of BCG.

Studies of the mechanism of cell-mediated tumor graft rejection have continued. We have found that skin inflammed by skin reactive factor does not support tumor growth (study performed with Dr. Daniel Thor, Division of Biologic Standards). This observation is in agreement with other observations made in this laboratory that suggest that tumor rejection occurs by a sequence of events. The first step is the specific recognition of the tumor cell by sensitized lymphocytes. Lymphocytes after recognizing tumor antigens elaborate products such as skin reactive factor and macrophage migration inhibitory factor. These factors lead to the accumulation of monocytes at the tumor site. Tumor cell death results from the interaction of lymphocytes and monocytes with tumor cells.

We have investigated why antigenic tumors grow progressively. One series of experiments indicates that animals have impaired ability to accept transferred tumor immunity and to generate an inflammatory response to a variety of irritants. Furthermore animals with large intramuscular tumors do not express delayed hypersensitivity _in vivo_ to protein antigens but do display delayed hypersensitivity _in vitro_ to these antigens. The observations suggest that one defect present in advanced neoplasia is a decreased number of monocytes capable of responding to immunologically specific or nonspecific irritants. This defect may explain the impaired ability of these animals to accept transferred tumor immunity. A second series of experiments indicates that lymphocytes that recognize tumor cells are specifically inactivated by the tumor. These two lines of investigation indicate that impairment of both lymphocytes and monocytes occurs in neoplasia and may account in part for the failure of the host to reject antigenic tumors.

A collaborative project with Dr. Michael Hanna, Jr., Carcinogenesis Area, Oak Ridge National Laboratory has been started to investigate the histopathology of the regression of established tumors at sites of delayed hypersensitivity reactions. Particular attention is being given to alterations in lymph node morphology. We have found, 1) histologic evidence of metastases in the regional lymph node 7 days after intradermal inoculation of tumor cells, 2) increased numbers of tumor cells in the regional lymph nodes of animals treated with intratumor injection of BCG and 3) sinus histiocytosis present in the regional lymph nodes of guinea pigs treated with intratumor injection of BCG.

Studies have been initiated in collaboration with Dr. T. Crandall Alford, George Washington University Hospital, to determine if human tumors regress at sites of delayed hypersensitivity reactions to BCG. We have found that this treatment leads to regression of cutaneous metastases of breast cancer. In collaboration with Dr. Donald Schwartz, Viral Leukemia and Lymphoma Branch, we have found that oncogenesis by the Moloney sarcoma virus is inhibited at the site of delayed hypersensitivity reactions to BCG.

Immunochemistry Section. Members of the Immunochemistry Section continue to elucidate the humoral aspects of immunity, especially cytotoxic reactions due to antibody and complement and to attempt the identification of antigens with the help of humoral immune factors. Major advances have been made in our understanding of the mechanism of complement action in the lysis of cells. We showed that under certain conditions each single complement-fixing antibody on the cell surface generates hundreds of lytic sites ("clusters"). These clusters amplify the cytotoxic effects of antibody-antigen complexes on cell surfaces. Our studies on the binding of the third component (C3) of complement to cells and antibody-antigen complexes confirm the presence of clusters of complement components on cell surfaces. We also found that C3 taken up on cell surfaces may appear in different forms depending on the antibody-antigen complex that initiated the fixation of complement. Because this component is pivotal in many of the reactions due to complement (cytotoxic, chemotactic, immune adherence, phagocytosis) the finding of different forms of C3 on cells may elucidate the mechanism of inhibition and amplification of cell damage by complement.

The interaction of specific antibody and complement with target cell surface antigens leads to the formation of lesions that permit the loss of intracellular cations, free amino acids, RNA but not DNA. The effect of immune cytolysis on the structural integrity of DNA was studied. We found that immune cytotoxic damage to Chinese hamster lung cells in culture leads to enzymatic degradation of target cell DNA, which resulted in greater than tenfold reduction in molecular weight of single stranded DNA fragments. The amount of DNA degradation was a function of antiserum concentration and correlated with lethal cell injury as measured by loss of colony forming ability.

Because complement components can be measured with exquisite sensitivity and accuracy we have continued our efforts to identify the cells that produce complement components. We have identified the mononuclear macrophage as the major source of the second and fourth components of complement, and we found that some cells are capable of synthesizing both components. In collaboration with other laboratories we have screened many permanent tissue culture lines including so-called permanent liver cultures. Some of these lines have been screened for synthesis of C4 and C2 before and after the application of carcinogens. Although interpretation of these data is most difficult, they show that carcinogens may profoundly affect in vitro synthesis of complement components and that cell lines that have been thought to be of liver cells may in fact be lines of macrophages. Thus we believe that because of the sensitivity, specificity and accuracy of complement component measurement these components can serve as biochemical markers in studying *in vitro* and *in vivo* effects of carcinogens, antimetabolites and other biochemicals on pathways of protein synthesis.

We are in the process of starting a contract to study in detail the effect of carcinogens on the *in vitro* production of complement components. In connection with these studies Cordis Corporation under contract is studying the *in vivo* effects of carcinogens on complement components. A major finding is that diethylnitrosamine fed to guinea pigs in carcinogenic doses profoundly reduces the C4 content of serum long before the appearance of tumors; this reduction is not due to direct interaction of the carcinogen and C4 and is not due to immediate toxic effects.

Considerable time is now expended on the solubilization and measurement of tumor antigens induced by chemical carcinogens (see report of Molecular Separations Unit).

Serial No. NCI-4662
1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immune Cytolysis

Previous Serial Number: Same

Principal Investigator: Harvey R. Colten, M.D.

Other Investigators: Tibor Borsos, Sc.D.

Cooperating Units: None

Man Years
Total: 0.0
Professional: 0.0
Other: 0.0

Project Description

Objectives:

To study the cellular and subcellular effects of antibodies and complement on nucleated cells in tissue culture.

Methods Employed:

Immune cytolysis is quantitated by colony inhibition. Antibodies against the cells are detected by the C1 fixation and transfer test. Changes in target cell DNA following immune cytolysis are analyzed ultracentrifugally on alkaline sucrose density gradients.

Major Findings:

Due to the lack of personnel, this project is temporarily suspended.

Significance to Biomedical Research and the Program of the Institute:

The elucidation of *in vitro* effects of cytotoxic agents on cells and subcellular components is important in the understanding of normal biological control mechanisms. The possibility of losses of these controls in the development of tumors or in the course of treatment of tumors with immunosuppressive agents may be studied *in vitro*. Furthermore, some of these findings, particularly with regard to DNA degradation may be of importance in understanding of autoimmune diseases.

Proposed Course of Project:

The method should be extended to investigating the effects on these target and other types of target cells of additional humoral and cellular cytotoxic factors.

Publications

Shipley, W.U., Baker, A.R. and Colten, H.R.: DNA degradation in mammalian cells following complement-mediated cytolysis. *J. Immun*., 106: 576-579, 1971.

Serial No. NCI-4663
1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Interaction of Beta-1C Globulin with Antigen-Antibody Systems

Previous Serial Number: Same

Principal Investigator: Edward J. Leonard, M.D.

Other Investigators: Tibor Borsos, Sc.D.

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

The measurement of beta-1C on cell surfaces has potential as a sensitive detection system for very low concentrations of antibody. We have also developed an inhibition assay so that beta-1C globulin on any solid phase can be measured.

Methods Employed:

Sheep red cells, appropriate antisera, lytic tests and the C1 fixation and transfer test were used to develop systems described below.

Major Findings:

We have completed development of a sensitive and practical test for measurement of C3 bound to a solid phase. The testing reagent is a supply of sheep erythrocytes to which C3 is bound (EC3) by the reaction scheme outlined in last year's report. When EC3 are reacted with anti-C3 antibody complement is fixed and can be detected quantitatively by either (1) the C1 fixation and transfer test or (2) the amount of lysis which occurs when whole complement is added to the EC3-anti-C3 complex. The hemolytic activity of anti-C3 antibody is inhibited by fluid phase C3 or by C3 bound to immune complexes. The quantitative test for bound C3 antigen is based on the inhibition of anti-C3 antibody by C3. The C3 to be measured (in either bound or fluid phase form) is added to a standard amount of anti-C3 antibody. Residual antibody activity is then determined by adding the absorbed antiserum to sheep EC3 indicator cells and measuring lysis of the cells which occurs after addition of whole guinea pig complement. By comparing the observed lysis with an inhibition-of-lysis curve obtained with known C3 antigen, the amount of test antigen can be estimated. The method is simple, rapid and reproducible and can detect nanogram amounts of bound C3.

Using the new assay, we have measured bound C3 in many particulate systems including erythrocytes, leukocytes, agarose beads, viruses and tumor cells. We have quantified the amount of C3 bound to the erythrocytes of patients with positive non-gamma Coombs tests. Since the ratio of bound C3 to bound antibody is at least 100/1 in several of the systems studied, we conclude that the bulk of the C3 must be bound to cell surface rather than antibody. We have also found that C3 taken up on cell surfaces may appear in different forms depending on the antibody-antigen complex that initiated the fixation of complement.

Significance to Biomedical Research and the Program of the Institute:

Since C3 is pivotal in many of the reactions due to complement (cytotoxic, chemotactic, immune adherence, phagocytosis) the finding of different forms of C3 on cells may elucidate the mechanism of inhibition and amplification of cell damage by complement.

Proposed Course of Project:

We plan to pursue our finding that C3 can be bound in different forms, using the action of C3 inactivator as a reflection of the differences.

Publications

Askenase, P. W., and Leonard, E. J.: C3 in high molecular weight complexes: Separation from plasma of nephritic patients and normal subjects by a method that avoids *in vitro* artifacts. *J. Immun*., in press.

Borsos, T., and Leonard, E. J.: Detection of bound C3 by a new immunochemical method. *J. Immun*., in press.

Serial No. NCI-4667
1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Isolation and Study of Tumor-Specific Antigens

Previous Serial Number: None

Principal Investigator: Edward J. Leonard, M.D.

Other Investigators: Monte Meltzer, M.D., Herbert J. Rapp, Sc.D. and Tibor Borsos, Sc.D.

Cooperating Units: Office of the Chief, Biology Branch and Immunochemistry Section, Biology Branch Etiology, NCI

Man Years
Total: 1.5
Professional: 1.5
Other: 0.0

Project Description

Objectives:

To obtain tumor-specific antigens in soluble and partially purified form. To use these antigens in inducing, establishing and measuring tumor specific immunity in guinea pigs. Knowledge derived from the guinea pig model will be applied to the isolation, purification and characterization of human tumor specific antigens. These antigens will be then used in diagnostic studies of human tumor epidemiology, in monitoring patient response to treatment and as an aid in treatment. Initial studies will be on the ascites cell form of diethylnitrosamine induced hepatoma of strain-2 guinea pigs.

Methods Employed:

Since histocompatibility antigens have been obtained in soluble form by extraction of cells with concentrated KCl solutions, we used the same approach for tumor cell antigen extraction. We extracted the ascites form of line 1 tumor cells originating in strain 2 guinea pigs and tested for antigen by the capacity of the extract to induce delayed skin reactions in strain-2 guinea pigs immunized to the line 1 tumor. We applied other techniques of protein fractionation (such as chromatography, ultracentrifugation, etc.) to purify the extracts, using the skin test to identify antigen-rich fractions.

Major Findings:

We have extracted line 1 tumor specific antigen from living line 1 ascites cells or from lyophilized cells. The initial yield of antigen is about 10%, based on comparison with the ability of living cells to induce a positive skin test. The 10% yield is a conservative estimate since it does not take into account the capacity for living cells to multiply at the skin test site. Further purification of antigen without significant loss has been achieved by low ionic strength precipitation of non-antigen protein, precipitation of antigen by 2M ammonium sulfate and gel filtration on Sephadex G-200. The bulk of antigenic activity is eluted between 50 and 75% of the Sephadex G-200 bed volume, suggesting that the molecular weight limits are less than IgG and more than serum albumin. The fact that the antigen entered the gel beads and was eluted in a virtually identical pattern in two runs on different length columns indicates that the antigen is soluble and probably homogeneous. It is stable for weeks on storage at 4°C or in the frozen state.

Significance to Biomedical Research and the Program of the Institute:

The stability of the isolated antigen suggests that it would be possible to obtain it in a highly purified form suitable for studies on its chemical nature and for induction of specific antibody. Purified antigen and monospecific antibody would provide opportunities to experiment with anti-tumor vaccines, to detect development of antigen in tissue culture carcinogenesis models and to study host responses against neoplastic cells.

Proposed Course of Project:

Studies are in progress on the capacity of the soluble antigen to induce tumor immunity and to stimulate antibody production. The ability of the antigen to cause transformation of lymphocytes immune to line 1 tumor is also under investigation. We are planning to attempt extraction of human tumor-specific antigen, using autologous skin testing as an antigen assay.

Serial No. NCI-4668
1. Biology Branch, OASDC, Etiology
2. Molecular Separations Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cardioglobulin

Previous Serial Number: None

Principal Investigator: Edward J. Leonard, M.D.

Other Investigators: Stephen Hajdu, M.D., Laura Stewart, Ph.D. and Glennwood Trivers, B.S.

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism, NHLI

Man Years

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description

Objectives:

To develop immunochemical assay methods for free and cell-bound cardioglobulin; to apply these methods to the isolation and purification of cardioglobulin components; and to look for cardioglobulin alterations in disease.

Methods Employed:

Isolated frog hearts were used for binding and bioassay of rat cardioglobulin-C. Immunochemical and immune fluorescene methods were employed in an attempt to identify cardioglobulin-C antigen. Standard techniques of protein fractionation were used to separate cardioglobulin-C antigen from other serum proteins.

Major Findings:

A. Reagents for immunochemical assay of rat cardioglobulin-C. Development of an assay is based on two reagents: (1) A BC-frog heart. This is an isolated and perfused heart exposed first to diluted human serum, then to a Sephadex G-200 fraction of rat serum. After Ringer solution wash, the heart retains one rat protein on its surface, rat cardioglobulin-C. (2) A multivalent rabbit antiserum to rat serum proteins including rat cardioglobulin-C. Antibody to rat cardioglobulin-C in this preparation fixes to rat C on the BC-heart. The fixation can be detected with fluorescein-labelled anti-rabbit serum.

B. Pedigree of anticardioglobulin antiserum. In previous work it was found that: 1. The antibody binds to surface membrane cardioglobulin-C sites of a BC-frog heart. This is detected by fluorescene. 2. The antibody inhibits the biological activity of cardioglobulin-C on the BC-heart. 3. The antibody binds to cell surface or basement membrane sites of many tissues of the normal rat; no antibody is detected if the serum is previously absorbed by a series of BC-hearts. 4. This year we have shown that the antiserum makes 3 precipitin lines when reacted with whole rat serum. After absorption with BC-hearts, precipitin line 3 is no longer detected. Our tentative conclusion, therefore, is that rat cardioglobulin-C is in precipitin line 3.

C. Fractionation of rat cardioglobulin-C. From the above findings, two immunochemical assays can be used to determine presence of rat cardioglobulin-C in a protein fraction. One is formation of precipitin line 3 by the fraction; the other is loss of antiserum capacity to fix to cardioglobulin-C sites in rat tissues or BC-hearts after absorption of the antiserum by a cardioglobulin-C fraction. Using these methods, we have found that the line 3 antigen appears to be remarkably heterogeneous by several fractionation criteria (gel filtration, sucrose density gradient ultracentrifugation and DEAE chromatography). However, only a restricted fraction of the line 3 antigen can abolish the capacity of anti-cardioglobulin antiserum to fix to tissue sites. It is likely that this fraction represents intact cardioglobulin-C.

Significance to Biomedical Research and the Program of the Institute:

Cardioglobulin is the name given to a system of proteins found in mammalian blood plasma. The protein system increases contractile force of isolated frog heart; the system is identified and its biological activity measured by using the frog heart as a bioassay. Cardioglobulin increases frog heart contractile force by transporting calcium into the cell interior. We hope to determine the functional significance of cardioglobulin starting with two clues: (1) Cardioglobulin is localized to cell membrane or basement membrane regions of many tissues; (2) the biological activity of cardioglobulin is profoundly decreased in systemic lupus erythematosus, a disease characterized by inflammatory lesions of many tissues and organs. Our working hypothesis is that cardioglobulin is a fundamental regulator of cell function, operating through its capacity to control trans-membrane calcium movement. Since calcium has been implicated in both cellular motility and cell-to-cell interaction, we are trying to determine whether cardioglobulin has a detectable role in such functions.

Proposed Course of Project:

Further purification of cardioglobulin components, production of monospecific antisera to these components and use of the antisera for studies of free and tissue bound cardioglobulin in human disease and experimentally induced cancer in rats.

Serial No. NCI-4629
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Mechanism of Cell Transformation

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Yuki Oshiro, Ph.D.

Cooperating Units: None

Man Years:	
Total:	3.7
Professional:	1.5
Other:	2.2

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for the quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.
2. Determination of ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.
3. Development of assays to determine whether *in vitro* transformation is accompanied by the appearance of tumor specific antigens.
4. Determination of the role of non-oncogenic and oncogenic viruses in chemical transformation.
5. Determination of the ultrastructural changes of cells during the course of *in vitro* chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

Methods Employed:

All procedures performed are with the view of quantitating phenomena *in vitro*. Such procedures are required in order to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with *in vitro* transformation. Cells used come from freshly isolated cells from animals that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with non-transformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole animal embryos or may be from specific organs. The cells are exposed to chemical carcinogen either prior to or subsequent to seeding the cells in the petri dish.

Approximately one week subsequent to treatment the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity, and spectrum of morphology of both the normal and transformed colonies. The frequency of transformation is expressed in a number of different ways. These take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

Major Findings:

Studies carried out during the past year have been concerned with the following:

1. Extension of the observation that transformed cells give rise to cell lines that produce tumors.
2. Determination of additional classes of chemical carcinogens which produce transformation.
3. Determination of structure-activity relationships among polynuclear hydrocarbons.
4. Effect of x-irradiation on susceptibility of cells to transformation by benzo(a)pyrene.

Transformations obtained as the result of 3-methylcholanthrene, benzo(a)pyrene, and 7,12-dimethylbenz(a)anthracene were studied in respect to morphology, oncogenicity and karyological characteristics. The patterns of various cell lines when produced by the same carcinogen differed in terms of types of cells present as well as degree of altered growth pattern. Cloning experiments resulted in the formation of colonies which usually had more than one type of morphological transformation. Cells of established lines derived from each transformed culture resulted in tumor formation when tested by subcutaneous inoculation into hamsters. No tumors were produced when control cells were inoculated into irradiated animals. Characteristic of the transformed cell lines analyzed for karyological changes was the low incidence of chromosomal markers. The cell lines, regardless of carcinogen used for transformation, had chromosome modes which were classified as hypodiploid or hyperdiploid;

the tumor derived cultures were quite similar to the transformed cell line in chromosome mode except for two which had shifted to the hypotetraploid range. The lack of common karyotopic changes demonstrates that the alterations were trivial and represent a random occurrence which is independent of the transformation. Since the transformed colonies were obtained only with carcinogens and were responsible for cell lines which produced tumors when injected into hamsters whereas control untransformed cell lines failed to produce tumors, it is concluded that carcinogenesis can be studied by an in vitro model with a high degree of reliability and that in vitro studies are relevant to in vivo neoplasia.

The ability of our bioassay system to recognize carcinogens belonging to other than the polycyclic hydrocarbon class has been investigated. Transformations have been observed after treatment with 11-methylcyclopenta(a)phenathen-17-one, aflatoxin B_1 N-hydroxy-N-2-fluorenylacetamide, and N-acetoxy-N-2-fluorenylacetamide. Single treatments with urethane as high as 50 μg/ml and diethylnitrosamine (100 μg/ml) failed to cause transformation. However, single injections of diethylnitrosamine to 12-day pregnant hamsters 48 hours prior to killing produced transformed colonies when fetal cells were plated from a secondary culture.

Structure activity relationships among some polynuclear hydrocarbons has been determined. We have found that 1-keto-3-methylcholanthracene, 3-hydroxybenzo-(a)pyrene, 7-hydroxymethyl-12-methylbenz(a)anthracene and 12-hydroxymethyl-7-methylbenz(a)anthracene all known to be active as local carcinogens had the ability to transform hamster fetal cells. The extent of activity correlated extremely well with known in vivo carcinogenic action. The two dimethylbenz(a)-anthracene derivatives were much less toxic than the parent compound. Whether these compounds including the 1-keto-3-methylcholanthrene are involved in the carcinogenicity of the parental compounds remains to be seen. Excellent correlation has been obtained with a series of four compounds which were reported by Dr. Saffiotti and associates as being carcinogenic for mice. These dihydro-compounds, 5,6-dihydrodibenz(ah)anthracene, 5,6-dihyrodibenz(aj)anthracene, 8,9,10,11-tetrahydro-7,12-dimethylbenz(a)anthracene and 5,6-dihydro-7,12-dimethylbenz(a)anthracene, gave results which reflected the known in vivo potency. Their transformation ability was rated from weak to strong.

To investigate the effect of irradiation of benzo(a)pyrene (BP) transformation, monolayer cultures were irradiated, plated for cloning, and treated with carcinogen at different intervals. Cells were exposed to either 150, 250, 500 or 1000 r of x-ray using a 200 kv Westinghouse machine. The cloning efficiency of irradiated only cells relative to unirradiated cells and of irradiated plus carcinogen treated cells relative to irradiated only cells was plotted for the different dosages of radiation used. It can be concluded that cell survival at the various radiation exposures tested was not affected by the addition of benzo(a)pyrene subsequent to radiation. Enhancement of transformation by radiation occurred at all dosages of radiation but was most pronounced with 500 r. The peak effect occurred at 48 hours with either 2.5 micrograms or 10 micrograms of BP/ml medium and was respectively approximately 8 and 5 times greater than BP alone. The current study supports the concept that irradiation selects cells more likely to be transformed by chemical carcinogen, implying that the cells were so modified that there was an increase probability that they would undergo transformation in the presence of carcinogen.

Significance to Biomedical Research and the Program of the Institute:

The need to study chemical carcinogenesis *in vitro* is obvious when one realizes that carcinogenesis is really a problem in cell ecology. Consequently, *in vitro* carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of *in vivo* problems. Therefore, *in vitro* carcinogenesis has wide applications in terms of studying compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course:

It is planned to further study the conditions which are responsible for or can alter *in vitro* transformation. These will include the use of x-rays, combinations of carcinogens of different classes, the combination of *in vivo*-*in vitro* systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. In addition, experiments are planned to test whether neoplastic transformation has occurred as a result of direct induction by chemicals, as the result of selection or of viral activation. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

Honors and Awards

First Congress of the European Association for Cancer Research.

Talk - Xth International Cancer Congress, Panal 38, Houston, Texas, 1970, Joseph A. DiPaolo.

Publications

Connell, D. I., Riechers, L. A., and DiPaolo, J. A.: Radioautographic analysis of 7,12-dimethylbenz(a)anthracene-^{3}H. Incorporation and cell survival of Syrian hamster embryo cells during exposure to nucleic acid inhibitors. *J. Nat. Cancer Inst.* 46: 183-193, 1971.

DiPaolo, J. A., Nelson, R. L., and Donovan, P. J.: Characteristics of primary tumors induced by carcinogenic polycyclic hydrocarbons in Syrian hamsters. *J. Nat. Cancer Inst.* 46: 171-181, 1971.

DiPaolo, J. A., Donovan, P. J., and Nelson, R. L.: *In vitro* transformation of hamster cells by polycyclic hydrocarbons: Factors influencing the number of cells transformed. *Nature*, In Press.

DiPaolo, J. A.: Chemical carcinogenesis *in vitro*. *Extrait de Acta Union Inter. Contre le Cancer*, In Press.

Serial No. NCI-4631
1. Biology Branch, OASDC, Etiology
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental Mammalian Teratogenesis and its Relationship to Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.1
Other:	0.5

Project Description

Objectives:

To determine the extent to which carcinogenic compounds are teratogenic and the extent to which teratogenic compounds are carcinogenic. To determine whether teratogenicity can be used as an index of carcinogenicity, and therefore, whether a short-term teratogenic experiment can be used to predict which compounds should be studied for long-term carcinogenesis. Finally, to study the physiological bases of teratogenic compounds and their possible relationship to the processes of carcinogenesis.

Methods Employed:

Mice, rats and hamsters are used in the studies. Careful record is kept of the gestational stage at which the teratogen is applied so that critical periods are established. Both single and multiple generation reproduction studies are being made to evaluate fertility of male and female ova implantation, embryonic and fetal development, resorption, abortion, delivery, live births, litter size, teratogenic effects (both macroscopic and microscopic), viability of new born, and maturation of young.

Major Findings:

To minimize the maternal influence of fetal-drug interaction, the compound must be applied directly to the embryo *in utero* or *in vitro*. The culture procedure has been limited by the period of time embryos may be kept alive in culture; the intra-aminotic injection has been limited to older embryos. Although 6-aminonicotinamide is a teratogen in chickens, mice, rats and rabbits, ours is the first report describing its teratogenic activity in hamsters. Hamster embryos were injected intra-amniotically (ia) with (6-AN) on gestation day 7, 8 or 9 and examined near term. Control treated embryos received 5 µl of water, a needle puncture without injection, or no treatment, and had low rates of resorption and malformation. 6-AN injected ia produced offspring with multiple defects including exencephaly, cleft palate, anophthalmia, micrognathia, and hydrocephalus. In contrast to day-8 and -9 embryos, which responded to 5-µg doses of 6-AN, day-7 embryos required 50-100 µg before exhibiting abnormalities. Thus, it is possible to study the influence of agents administered directly to embryos during pre-and early-somite stages.

The potential teratogenic effect of cyclamate, cyclohexylamine and saccharin were investigated using 8-day pregnant S hamsters. All compounds were injected once with the LD_{50} dose. These compounds had little influence on production of malformations.

Significance to Biomedical Research and the Program of the Institute:

There is evidence, both clinical and experimental, of a relationship between teratogenesis and carcinogenesis. When one compound is both a carcinogen and a teratogen, it is important to determine whether the mechanism of action is similar so that the mechanism of chemical teratogenesis may be considered in elucidating the mechanism of chemical carcinogenesis.

Proposed Course:

This project has been terminated because of lack of personnel.

Honors and Awards

Lecture -"Parallelism between carcinogenesis and teratogenesis", Joseph A. DiPaolo, George Washington University Medical School.

Publications

Turbow, M. M., Clark, W. H., and DiPaolo, J. A.: Embryonic abnormalities in hamsters following intrauterine injection of 6-aminonicotinamide. *Teratology*, In Press.

Serial No. NCI-4664
1. Biology Branch, OASDC, Etiology
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunologic Approaches to the Prevention and Treatment of Cancer

Previous Serial Number: None

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Tomiko Tanaka, Ph.D., Irwin D. Bernstein, M.D., Gerald L. Bartlett, M.D., and Tibor Borsos, Sc.D.

Cooperating Units: For supply of BCG: Trudeau Institute, Saranac Lake, New York. For studies of the histopathology of BCG-mediated tumor regression: Dr. M. Hanna, Jr., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. For studies of murine sarcomas, Dr. D. Schwartz, Viral Leukemia and Lymphoma Branch, NCI. For studies of women with breast cancer: Dr. W. Terry, Immunology Branch, NCI and Dr. T. C. Alford, George Washington University Hospital.

Man Years:

Total:	4.0
Professional:	2.0
Other:	2.0

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods suited to the prevention and treatment of cancer. This objective is based on the finding that tumors contain unique antigens capable of eliciting the formation of tumor specific humoral antibodies and of causing the appearance of cells which react specifically with tumor antigens. Transplantable syngeneic guinea pig tumors will be used to investigate conditions for effective immunotherapy. Murine sarcoma virus of Moloney will be used to investigate prevention of viral oncogenesis.

Methods Employed:

Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine (DEN) in their drinking water. Seven of these tumors are being maintained by periodic intramuscular transplantation. Ascites variants have been derived from five of these transplantable tumor lines. Two of these ascites variants are being maintained by periodic intraperitoneal transplantation. A strain-2 guinea pig leukemia is also being maintained by periodic intradermal transplantation. Portions of these tumors are periodically frozen and stored in liquid nitrogen. The work in this report was carried out with cells of a transplantable hepatoma (line 10) which when injected into the skin produce a progressively growing papule and regional lymph node metastases within a few days.

Animals are immunized to mycobacterial antigens by intradermal injection of living Mycobacterium bovis (strain BCG). 28 to 35 days after immunization, guinea pigs are inoculated intradermally with living tumor cells alone or with a mixture of living tumor cells and BCG. In some experiments immunized guinea pigs receive tumor cells alone into one flank and BCG alone into the contralateral flank. The papule produced by injection of living tumor cells or a mixture of living tumor cells and BCG is measured at intervals following injection. Delayed hypersensitivity reactions are measured 24 hours after inoculation of antigen.

The viability of tumor cell suspensions exposed to living BCG is measured by trypan blue exclusion and by incorporation of tritiated thymidine into tumor cell DNA.

Tumor immunity is measured by the response of guinea pigs to an intradermal challenge with living tumor cells. Bacterial immunity is measured by the response of guinea pigs to an intradermal injection of heat-killed BCG.

Optimal conditions for immunotherapy of a transplantable hepatoma have been evaluated. Guinea pigs are immunized to BCG as described. Tumor cells are inoculated intradermally 28-31 days after immunization. Living BCG is inoculated into the intradermal tumor 7 or 12 days after tumor implantation.

Intradermal inoculation of a mixture of living BCG and living tumor cells into one flank and of tumor cells alone into the contralateral flank on the same day can be used to study immunotherapy.

Murine sarcoma virus of Moloney is inoculated intramuscularly into mice immunized to BCG or into nonimmunized mice. Some mice receive MSV alone; other mice receive a mixture of MSV and BCG.

Major Findings:

1. Complete tumor inhibition was observed when infection with living BCG occurred at the site of tumor inoculation.

2. This inhibition of tumor growth was not attributable to a direct cytotoxic action of living BCG on tumor cells.

3. Inhibition of tumor growth was mediated by the host in the course of an immunological response of the delayed hypersensitivity type to the infecting organisms.

4. Animals which rejected tumor cells at the site of an infection with BCG showed suppressed tumor growth and delayed hypersensitivity reactions to a subsequent challenge of tumor cells.

5. The minimum number of living BCG capable of eliciting a host response that would completely kill tumor cells was 6 x 10^6 organisms in immunized and 6 x 10^5 organisms in unimmunized animals.

6. Optimal suppression of tumor required living BCG, close contact between tumor and BCG, and a cell-mediated immune response by the host.

7. Intradermal inoculation of cells of a transplantable ascites hepatocarcinoma leads to the formation of a progressively growing intradermal tumor with regional lymph node metastases.

8. Metastases are present by 6-7 days after intradermal tumor implantation as shown histologically and by transplantation of lymph nodes into guinea pigs.

9. Surgical excision of the intradermal tumor 6-8 days after inoculation, or local irradiation of the intradermal tumor 8 days after inoculation while locally effective do not prevent the metastases from growing and killing the host.

10. Active immunization against tumor specific transplantation antigens of the tumor is also ineffective.

11. Inoculation of living BCG organisms into the intradermal tumor causes local tumor regression and prevents the growth of metastases.

12. Histologic studies suggest that histiocytes are of major importance in killing lymph node metastases. Sinus histiocytosis is present in the regional lymph nodes of guinea pigs treated with intratumor injection of BCG. Histiocytes are seen in contact with dying tumor cells.

13. Intradermal injection of a mixture of living tumor cells and living BCG produces a state of systemic immunity to the tumor. This vaccine is effective therapy for an animal with a relatively small tumor burden (10^5 to 10^6 tumor cells in an intradermal papule).

14. Oncogenesis by the murine sarcoma virus of Moloney is completely inhibited at sites of immunologic reactions of delayed hypersensitivity type to living BCG. Mice so treated resist a lethal intramuscular challenge with MSV.

15. Systemic tumor immunity was produced by intratumor injection of BCG.

16. Complete tumor regression was produced by intratumor injection of BCG in 75% of guinea pigs with tumor nodules 100 mgm in size and in 17% of guinea pigs with tumor nodules 400 mgm in size.

17. The limiting factor in effective immunotherapy appeared to be related to the number of lymph node metastases.

18. Cutaneous metastases of human breast cancer regress at sites of delayed hypersensitivity reactions to BCG.

Significance to Biomedical Research and the Program of the Institute:

Immunologic methods have been shown in man to be of value in the treatment of epidermal tumors, intradermal metastases of malignant melanoma and acute leukemia. Successful treatment of skin tumors in man requires the development of an inflammatory reaction of the delayed type at the site of the tumor. Our work provides an animal immunotherapy model based on principles shown to be of value in man. This model may be conveniently used for experiments designed to resolve the question of the nature of the mechanism of immunologic tumor destruction and the question of whether established tumor in sites other than the skin can be controlled by this kind of immunotherapy. These experiments provide a firm experimental basis for clinical trials.

Results suggest that it may be possible to produce strong tumor immunity to "weakly" antigenic tumors by injecting the living tumor cells together with living BCG. This finding suggests an additional method for inducing tumor specific transplantation immunity.

The demonstration that viral oncogenesis can be inhibited at sites of delayed hypersensitivity reactions may lead to a better understanding of the molecular and cellular events controlling viral and chemical oncogenesis.

Proposed Course:

Immunotherapy experiments will be directed to determine whether tumors at sites other than the skin can be controlled by delayed hypersensitivity reactions to antigens unrelated to tumor antigens.

Attempts will be made to improve the effectiveness of the current BCG-tumor cell vaccine. The form of mycobacterial antigen, the form of tumor cell antigen will be varied. The effects of pertusses vaccine will be evaluated.

Studies of women with breast cancer will continue. Efforts will be made to establish an *in vitro* or *in vivo* test that measures tumor specific immunity. The effect of intratumor injection of BCG on tumor specific immunity will be tested.

Publications

Zbar, B., Bernstein, I. D., Tanaka, T., and Rapp, H. J.: Tumor immunity produced by the intradermal inoculation of living tumor cells and living *Mycobacterium bovis* (strain BCG). *Science* 170: 1217-1218, 1970.

Zbar, B., Bernstein, I. D., and Rapp, H. J.: Suppression of tumor growth at the site of infection with living Calmette-Guérin Bacillus. *J. Nat. Cancer Inst.*, In Press.

Zbar, B., and Tanaka, T.: Immunotherapy of cancer: Regression of established tumors after intralesional injection of living *Mycobacterium bovis* (strain BCG). *Science*, In Press.

Wepsic, H. T., Kronman, B. S., Zbar, B., Borsos, T., and Rapp, H. J.: Immunotherapy of an intramuscular tumor in strain-2 guinea pigs: Prevention of tumor growth by intradermal immunization and by systemic transfer of tumor immunity. *J. Nat. Cancer Inst.* 45: 377-386, 1970.

Serial No. NCI-4665
1. Biology Branch, OASDC, Etiology
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanism of Delayed Hypersensitivity and Tumor Graft Rejection

Previous Serial Number: None

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Irwin D. Bernstein, M.D., Tibor Borsos, Sc.D. and Harold T. Wepsic, M.D.

Cooperating Units: For preparation of macrophage migration inhibition factor: Dr. D. Thor, Laboratory of Virology and Rickettsiology, DBS. For supply of complete Freund's adjuvant: Dr. S. Chaparas, Laboratory of Bacterial Products, DBS. For development of an in vitro assay for enhancing alloantibodies (macrophage migration test) in mice: Dr. P. Wright, Laboratory of Biology, NCI. For in vitro studies of tumor suppression: Dr. J. Oppenheim, Laboratory of Microbiology, NIDR.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

The main objectives of this project are, 1) to define the mechanism of the immunological rejection of antigenic tumors, and 2) to ascertain possible immunologic defects in tumor-bearing hosts to explain the progressive growth of an antigenic tumor.

The long-range objective is to develop methods of eliminating immunological defects in host animals to allow for optimal immune response to the tumor.

Methods Employed:

Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine (DEN) in their drinking water. Transplantable ascites and intramuscular variants of these tumors currently maintained in this laboratory are used. A strain-2 guinea pig leukemia is also maintained and used.

Animals are immunized by intradermal injections of living tumor cells. Certain tumors require surgical excision of the intradermal papule for immunization. Peritoneal exudate cells (PEC) from immunized guinea pigs are used for their ability to transfer immunity to nonimmune guinea pigs. PEC are mixed with tumor cells and then injected intradermally (local passive transfer) or PEC are injected into the heart (systemic passive transfer) of unimmunized recipients. The results of these transfers of immune cells were evaluated in normal guinea pigs as well as animals with intramuscular tumors, animals with ascites tumors, and animals with leukemia. The results of the systemic transfer of immunity with PEC which were preincubated *in vitro* with tumor cells was also evaluated. Serum obtained from normal and tumor-bearing hosts were incubated with PEC prior to systemic injection, or tumor cells prior to intradermal injection to evaluate the possible presence of factors which would inhibit the transfer of tumor immunity to normal guinea pigs.

Lymph nodes obtained from guinea pigs immunized to bacterial antigens are incubated *in vitro* with specific antigen. Supernatant fluids from these cultures are tested for their ability to induce skin reactions (skin reactive factor). The growth of tumor cells at the site of these skin reactions was evaluated.

Animals with intramuscular tumors are immunized to bacterial antigens. They are skin tested with the specific antigen and then their lymph nodes are obtained and incubated *in vitro* with specific antigen. The response of the lymphocyte is evaluated by the increase in DNA synthesis, and also by the ability of the supernatant fluids to induce skin reactions in normal guinea pigs. Animals with intramuscular tumors are also evaluated for their ability to respond to inflammatory reagents injected into the skin (turpentine) or intraperitoneally (mineral oil). Skin reactive factor is injected into the skin of these tumor-bearing animals. The subsequent skin reaction and the growth of tumor at these sites was evaluated.

Animals immunized to tumor antigens are injected intravenously with tumor cells. The animals are then challenged intradermally with tumor cells to evaluate any change in their tumor immunity.

Syngeneic mice are sensitized to another syngeneic strain. PEC from the sensitized strain are mixed with donor PEC, and the migration of these cells from a capillary tube onto a glass surface is evaluated (macrophage migration test). Enhancing alloantisera were added to such cells and the subsequent migration of the PEC evaluated.

Major Findings:

Studies on the Mechanism of Tumor Graft Rejection

1. Soluble products of specifically stimulated lymphocytes (skin reactive factor) induce a delayed inflammatory reaction in the skin of normal guinea pigs. This factor associates with macrophage inhibition activity. The reaction site consisted mainly of mononuclear cells.

2. Tumor cell growth is suppressed at the site of an inflammatory reaction induced by these soluble lymphocyte products.

3. These soluble lymphocyte products can increase the ability of nonimmune macrophages to kill tumor cells *in vitro*.

4. PEC from sensitized and donor allogeneic mice show inhibition of migration from a capillary tube onto a glass surface. Enhancing alloantisera specific for the donor target cell abrogate the inhibition of migration.

Studies of the Inability of the Host to Reject an Antigen Tumor

1. Systemic transfer with immune peritoneal exudate cells is nonspecifically impaired in animals with intramuscular tumors.

2. Animals with intramuscular tumor show a decreased inflammatory response to oil intraperitoneally, turpentine intradermally, and skin reactive factor intradermally.

3. Animals with intramuscular tumors show a decreased ability to suppress an antigenically unrelated tumor at the site of a nonspecific inflammatory reaction induced by skin reactive factor.

4. Animals immunized to BCG, and bearing intramuscular tumors showed, a) a minimal or absent skin test reaction following the injection of PPD, and b) normal *in vitro* reactivity of lymphocytes in the presence of PPD as measured by ^{3}H-thymidine incorporation and skin reactive factor production.

5. Animals with intramuscular tumors given bone marrow transfusions were still refractory to a systemic transfer of tumor immunity.

6. Animals with leukemia could be systemically passively immunized to another tumor.

7. Local passive transfer of tumor immunity was not impaired in animals with intramuscular tumors.

8. The administration of a large dose of a given (living or irradiated) tumor intraperitoneally to an unimmunized guinea pig abrogates a subsequent systemic passive transfer of immunity directed at the given tumor when the time interval between intraperitoneal injection of tumor and passive transfer is relatively short.

9. The incubation of the specific tumor cells with immune PEC prior to systemic injection abrogates the transfer of immunity.

10. The injection of tumor cells intravenously or intraperitoneally to animals which have been previously actively immunized to that tumor, can decrease the animal's immunity towards that tumor.

Significance to Biomedical Research and the Program of the Institute:

The observations that tumor graft rejection can be initiated by transferred sensitized lymphocytes or products of specifically stimulated lymphocytes which cause the local *in vivo* accumulation of nonimmune mononuclear cells are significant. These findings support the concept that sensitized lymphocytes initiate tumor graft rejection and that the reaction is amplified by the recruitment of nonspecific cells.

Tumor graft rejection was not initiated by passively transferred lymphocytes in tumor-bearing animals; actively immunized tumor-bearing animals showed impaired delayed hypersensitivity despite the presence of a normally functioning lymphocyte component. These observations are consistent with the idea that a functional nonspecific cellular component required for delayed hypersensitivity and tumor immunity is lacking in tumor-bearing hosts.

The observations that injections of tumor cells intraperitoneally or intravenously can specifically abrogate passive or active tumor immunity offers further insight into understanding the mechanism by which antigenic tumors grow progressively in the host.

An *in vitro* assay for enhancing antibodies using the macrophage migration test was developed. The findings showed that enhancing antibodies block production by the lymphocytes of the soluble mediators of cellular immunity.

Proposed Course:

Attempts will be made to restore the functionally deficient nonspecific cellular component in tumor-bearing hosts so that these animals can manifest delayed hypersensitivity and tumor immunity. To do this, various cell components as well as nonspecific stimulants of the immune system (as BCG) will be administered.

The mechanism by which this deficiency occurs in host animals will be explored. The kinetics of the nonspecific cellular component will be evaluated (turnover studies).

The relative importance of these nonspecific mononuclear cells in the rejection of early (small) tumors will be evaluated. The nonspecific component will be neutralized by 6-MP or quartz dust and the ability of animals given competent immune lymphocytes to reject a tumor graft will be evaluated. Germ-free and newborn animals which have a mononuclear cell defect will also be similarly evaluated.

The macrophage migration test will be used as an *in vitro* assay for tumor immunity and blocking antibodies in a mouse tumor system.

Publications

Bernstein, I. D., Wepsic, H. T., Zbar, B., and Rapp, H. J.: Tumor immunity: Impairment in tumor-bearing hosts. *J. Nat. Cancer Inst.*, In Press.

Wepsic, H. T., Bernstein, I. D., Zbar, B., Borsos, T., and Rapp, H. J.: Abrogation of passively transferred tumor immunity *in vivo* by antigenically related tumor cells. *J. Nat. Cancer Inst.* 46: 195-202, 1971.

Bernstein, I. D., Thor, D. E., Zbar, B., and Rapp, H. J.: Tumor immunity: Suppression of tumor growth *in vivo* initiated by soluble products of specifically stimulated lymphocytes. *Science*, In Press.

Bernstein, I. D., and Wright, P.: *In vitro* detection of enhancing antibody by the macrophage migration test. *Nature*, In Press.

Serial No. NCI-4666
1. Biology Branch, OASDC, Etiology
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Tumor Specific Immune Reactions in Mice

Previous Serial Number: None

Principal Investigators: Herbert J. Rapp, Sc.D., and Berton Zbar, M.D.

Other Investigators: Gerald L. Bartlett, M.D., and Irwin D. Bernstein, M.D.

Cooperating Units: For studies of serum factors in migration inhibition: Dr. P. Wright, Laboratory of Biology, NCI. For supply of mice and tumors: Institute for Cancer Research, Philadelphia, Pa. For supply of BCG: Trudeau Institute, Saranac Lake, New York. For radiation facilities, Radiation Branch, NCI

Man Years:	
Total:	1.5
Professional:	1.0
Other:	0.5

Project Description

Objectives:

A long-range goal of this project is to improve our understanding and control of the processes by which a cancerous individual, animal or human, may be able to protect himself from his tumor. An important feature of such processes is the ability of the individual to specifically interfere with tumor growth by means of immune mechanisms. Such tumor specific immune reactions tend to be relatively weak, even in experimental circumstances. The immediate objectives of the project are, 1) to study tumor specific immune reactions to ascertain those factors which impose limitations on the effectiveness of the immunity, 2) to study, both inductively and deductively, means of strengthening anti-tumor immune reactions, and 3) to develop methods for the study of tumor specific immunity *in vivo* and *in vitro*.

One successful approach to augmenting tumor immunity in guinea pigs has been to immunize with mixtures of tumor and BCG (*Mycobacterium bovis*) rather than with tumor cells alone; the immunizing tumor inoculum failed to grow progressively when mixed with BCG and strong tumor immunity resulted. This project has adapted this technique to a mouse tumor system to facilitate certain manipulations of the immune response and to permit comparison of the response of several tumors. The work was intended to determine what types of processes are required to prevent tumor growth at the site of a BCG injection, and how such an interaction affects the subsequent development of tumor immunity.

One of the potential routes to failure of tumor specific immune responses is known as immunological enhancement, whereby humoral antibodies, specific for tumor antigens, somehow interfere with effective tumor destruction by cellular components of the immune response. Effort has been directed towards adapting the macrophage migration inhibition test (a reliable in vitro test of cellular immune reactions) to reflect interference by serum factors.

Methods Employed:

A variety of mouse sarcomas of the C_3H strain are available; four have been used thus far. Three were induced in subcutaneous tissue, by 3-methylcholanthrene; one developed, without carcinogen treatment, during prolonged culture of connective tissue in a diffusion chamber. One of the chemically induced tumors is highly antigenic; it grows with difficulty in normal syngeneic mice but grows readily in pre-irradiated mice. Another MCA sarcoma grows slowly and has been moderately antigenic in previous tests. The third chemically induced tumor grows very rapidly, metastasizes to regional lymph nodes, and has not effectively immunized syngeneic mice in previous tests. The "spontaneous" sarcoma has an average growth rate and has been very weakly antigenic. Portions of the tumors are frozen and stored in liquid nitrogen. They are maintained by serial, approximately biweekly transplantation in syngeneic mice which have been immunosuppressed by thymectomy and 450 r of x-radiation.

The solid sarcomas are minced and digested with Pronase and DNAase. Appropriately diluted suspensions of the tumor cells are injected intradermally, either alone or after mixture and short (10-30 min) incubation with living BCG. Tumors are measured biweekly. After growth of primary tumor grafts, tumors are excised. Evidence of tumor immunity is obtained by comparing the growth of a second tumor inoculum in pretreated mice with growth of a similar inoculum in mice receiving control treatments. Evidence for delayed hypersensitivity to BCG is obtained by measuring the degree of swelling of the footpad 24 hours after injection of 10 γ of PPD.

Sublethal x-radiation (450 r) is used as an immunosuppressive agent, and its effects are prolonged by prior thymectomy of the mice during young adult life. Thymectomized (at 8 weeks of age), non-irradiated animals are presumed to be able to develop primary immunity and to express immunological memory normally. Mice immunized to BCG prior to irradiation are expected to retain some sensitivity to BCG but to be unable to develop primary immunity (as to tumor antigens). Nonimmunized, irradiated mice are presumed to be unable to respond immunologically to new antigens.

Peritoneal exudate cells of nonimmune mice or from mice immune to another inbred strain, are packed into small capillary tubes and incubated in tissue culture media overnight to allow migration of the contained macrophages. The additional presence of the target antigen in the cell pellet inhibits migration of macrophages from specifically immune animals but not from nonimmune animals. The effects of various sera are tested by incubating the target cells in the serum prior to addition of the immune peritoneal exudate cells.

Major Findings:

Processes Involved in Tumor Suppression at the Site of BCG Infection

1. All three mouse tumors tested have failed to grow when injected into BCG-immune mice as a mixture with living BCG. In two individual experiments, with two different tumors, suppression was incomplete; the factors contributing to this failure have not been defined.

2. Tumors alone are not suppressed if BCG is injected simultaneously at a different site.

3. Tumor cells do not die during the *in vitro* coincubation with BCG prior to injection.

4. Tumor cells mixed with BCG are only temporarily suppressed in nonimmune, irradiated mice. Thus nonimmune processes may play a minor, but not a sufficient role in tumor suppression.

5. Tumor cells mixed with BCG can be permanently suppressed in BCG-immune, irradiated mice. Thus immune reactivity to BCG is sufficient to prevent growth of tumor cells mixed with BCG.

6. Tumor cells mixed with BCG can be permanently suppressed in mice not previously immunized to BCG.

7. Tumor cells mixed with BCG can be permanently suppressed in mice which subsequently are shown to have no tumor specific immunity. Thus, tumor immunity is not necessary for suppression of tumor growth at the site of BCG infection.

8. Direct injection of BCG into established, growing tumors may lead to tumor regression. A group of slow-growing tumors (3.9 mm average diameter after 12 days of growth) regressed after BCG injection; a different preparation of the same tumor grew more rapidly (4.2 mm in 6 days) and failed to regress after BCG injection.

9. Direct injection of BCG into primary tumors (early or late chemically induced papillomas or chemically induced subcutaneous sarcomas) had no different effect on tumor behavior than did control procedures.

Effects of BCG and BCG-Tumor Mixtures on the Development of Tumor Immunity

1. Injection of BCG has no effect on the growth of a subsequent (21 days) or a simultaneous, contralateral injection of tumor cells, (primary tumor immunity) or on the ability of such a tumor growth to induce immunity to subsequent challenge (secondary tumor immunity).

2. With one tumor, animals injected with a mixture of BCG and tumor cells (tumor cells did not grow) consistently had less resistance to tumor challenge than did mice immunized with temporary tumor growth.

3. With a different tumor, injection of a mixture of BCG and tumor cells (only partial suppression of the tumor cells) induced strong immunity to challenge, while temporary tumor growth induced no resistance at all. Despite the strong immunity to challenge, tumors from the BCG-tumor mixture produced metastases as frequently as did tumors growing without admixed BCG.

Adaptation of Macrophage Migration Inhibition Test to Detect Inhibiting ("Enhancing") Effects of Serum

1. Whereas the migration of macrophages from immune donors was inhibited in the presence of allogeneic target antigen and normal serum, migration was normal if the target cells were incubated with immune serum prior to introduction to the immune peritoneal exudate cells.

Significance to Biomedical Research and the Program of the Institute:

The demonstration that BCG can promote nonspecific tumor killing in mice, as well as in guinea pigs, supports the general significance of the phenomenon. Furthermore, tumors with a variety of biological and antigenic characteristics are susceptible. The demonstration that the local tumor killing is not at all dependent on tumor specific immunity, leads to concentration on the nature of the nonspecific processes involved and to a study of agents capable of inducing such reactions. The fact that immunity to BCG is necessary (and sufficient) to effect tumor killing at a BCG site establishes the phenomenon as a form of immunotherapy, albeit nonspecific.

The tumor lines studied differed markedly in the degree to which mixtures of BCG and tumor cells induced tumor immunity. This suggests that BCG tumor cell immunization may not be universally effective in boosting tumor resistance, but should be applied only with responsive tumors.

The availability of an *in vitro* means of detecting tumor enhancing factors will allow a more complete analysis of antitumor immune responses and the reasons for their failure.

Proposed Course:

Experiments will attempt to improve the ability to "treat" established tumors by direct injection of BCG, and to compare the susceptibility of different tumors to this effect. This may provide insight into factors which will limit the success of this type of immunotherapy and may identify tumor characteristics which determine susceptibility to the effect. In order to bring the model more into line with the problems of treating naturally occurring tumors, attempts will be made to develop techniques and to identify agents which can produce tumoricidal delayed hypersensitivity reactions at remote, inaccessible tumor sites. Models for combining immunotherapy with other therapeutic modalities will be explored.

The effect of BCG and BCG-tumor mixtures on tumor immunity will be analyzed to determine differences in numbers and effectiveness of immune cells, shifts in the balance of cellular-humoral (enhancing) immune responses or differences in the kinetics of immune responses. Agents other than BCG will be studied to determine their comparative effectiveness in augmenting tumor immunity and in the hope of identifying agents complementary to BCG. In situations where BCG-tumor mixtures are effectively immunogenic, the practicability of substituting tumor cells killed by various means will be examined.

The macrophage migration inhibition assay for enhancement may be further refined. Adaption of a quantitative *in vitro* measure of tumor immunity, the ^{51}Cr-release method, would improve our ability to study tumor immunity, and might also be adaptable to the demonstration of enhancing antibody. A major need is a method to quantitate the number of specifically immune cells produced during an anti-tumor immune reaction (as the number of antibody producing cells can be determined during humoral immune reactions). The possibility of developing such a technique will be explored.

Serial No. NCI-4636
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Physico-Chemical Characteristics of Complement Components

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Felix G. Sassano, M.D., Harvey R. Colten, M.D. and Herbert J. Rapp, Sc.D.

Cooperating Units: Molecular Separations Unit, Biology Branch ET, NCI

Man Years
Total: 2.0
Professional: 1.5
Other: 0.5

Project Description

Objectives:

To isolate chemically and functionally pure serum complement components and to characterize the physico-chemical properties and structure functional relationships of these biologically active molecules.

Methods Employed:

Complement components are purified by a number of techniques including gel filtration, preparative ultracentrifugation (including the B XIV zonal ultracentrifuge), ion exchange chromatography, and a new technique for the separation of molecules based on differences in isoelectric point. The effects on structure and function of isolated complement components of a number of physico-chemical parameters, such as changes in ionic strength, trace metal content, temperature and osmolarity are studied with immunochemical and physico-chemical techniques.

Major Findings:

Guinea pig C1 in the euglobulin fraction of serum when dialyzed against 0.05M EDTA/Veronal buffered saline was resolved by DEAE chromatography into three subunits, C1q, C1r and C1s similar to those obtained from human serum by Lepow, et.al. (J. Exper. Med. 117, 983: 1963). Sedimentation constants for the guinea pig subunits were obtained by sucrose density ultracentrifugation. The S value for guinea pig C1q was 10.2-10.7; for C1r, 6.9-7.3 and for C1s, 4.3. When homologous guinea pig subunits or homologous human subunits were recombined or when guinea pig subunits were recombined with human subunits generating hybrid C1 molecules, the resultant hemolytically active C1 molecule had a sedimentation constant of about 12S. As S value of 16-18 was obtained, however, for purified guinea pig C1, for C1 in guinea pig euglobulin and for C1 in guinea pig euglobulin dialyzed against EDTA with subsequent removal of EDTA. The lower sedimentation constant (12S) for recombined C1qrs suggests two possibilities: 1) the C1 molecule generated from subunits has a shape different from the native C1 molecule, or 2) multiples of one or more of the subunits are present in the native C1 and not all of them are necessary for recombination.

Significance to Biomedical Research and the Program of the Institute:

Basic studies of chemical and functional relationships of complement components are directed toward an understanding of the mechanism of complement fixation in general, and have potential application to studies of mechanisms of host resistance to infectious disease and tumors. Specifically it is necessary to determine the factors which inhibit or enhance the immunologic response to antigens. In the case of complement mediated cytotoxic reactions, isolation of cytotoxic antibody, cell antigens and complement in chemically or functionally pure form permits study of controlled reactions in which an estimate of the importance of a variety of chemical and physical conditions can be made. Preparation of molecules which are hybrids of fragments from two species permits detailed analyses of the molecular structure of these proteins.

Proposed Course of Project:

Further examinations of the functional and structural properties of the guinea pig and human hybrid C1 molecules are in progress. With these hybrid molecules it should be possible to study *in vivo* some of the functional properties of these proteins.

Serial No. NCI-4638
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biosynthesis and Ontogeny of Serum Complement

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Harvey R. Colten, M.D., H. V. Wyatt, Ph.D. and Herbert J. Rapp, Sc.D.

Cooperating Units: None

Man Years

Total:	1.5
Professional:	1.5
Other:	0.0

Project Description

Objectives:

To identify the site or sites of synthesis of individual complement components by means of isolated organ and tissue culture, and to determine the effect of a number of chemicals (e.g. antibiotics, antimetabolites, carcinogens, etc.) on the synthesis of complement components; to identify and characterize serum complement components at various stages of embryonic and fetal development, and to identify the site or sites of complement synthesis in fetal tissue.

Methods Employed:

Guinea pig or human organs are isolated and maintained *in vitro* during the period of observation.

De novo synthesis is confirmed by including ^{14}C-labelled amino acids in the incubation media and counting the ^{14}C-labelled complement component with standard counting equipment.

In addition, localization of the cell types synthesizing complement in vitro are detected by a hemolytic assay performed in agarose gel.

Major Findings:

We have identified the macrophage as a cell type that synthesizes C2. We have now found that C4 is also synthesized in macrophages. 2.4% of peritoneal exudate (PE) macrophages produced C4 when the cells were obtained by the injection of casein or oil intraperitoneally; 12% of starch induced PE macrophages produced C4. With a modified Jerne plaque technique designed to detect *in vitro* synthesis of both C2 and C4, we found that about 0.1% of PE macrophages produced both C4 and C2 in the same cell. Studies on the effect of hydrocortisone on C2 synthesis showed that this hormone inhibited the *in vitro* production of C2. In collaboration with other laboratories we have screened many permanent tissue culture lines including so called permanent liver lines for the production of C4, C2 and C1. The data show that numerous lines of cells produced C4 or C2 or both in culture and that *in vitro* exposure of these cells to chemical carcinogens may profoundly alter complement synthesis. Furthermore, the data suggested that cell lines that have been thought to be of liver cells may be lines of macrophages.

Significance to Biomedical Research and the Program of the Institute:

Studies on the synthesis of complement components by isolated tissues and cells, because of the extreme sensitivity of the assays, provide some insight into the early events in the biosynthesis of proteins. In addition, the techniques used in this investigation provide a sensitive, rapid and easily performed assay for the effect of chemical and physical factors, including carcinogens, antibodies, steroids, ionizing radiation, etc., on the synthesis of a protein.

Proposed Course of Project:

The studies of complement synthesis and ontogeny of complement components in guinea pigs and sheep are now being extended to studies of human complement. Attempts to identify mechanisms controlling the synthesis of complement may provide an explanation for failures of host defense in a number of diseases, including patients with certain malignant diseases. The in house portion of this project will be phased out; it is planned to continue the effort extramurally.

Publications

Rubin, D.J., Borsos, T., Rapp, H.J., and Colten, H.R.: Synthesis of the second component of guinea pig complement in vitro. J. Immun. 106: 295-303, 1971.

Colten, H.R., and Wyatt, H.V.: Biosynthesis of serum complement. Symposium Proceedings of Canadian Society of Immunology, in press.

Serial No. NCI-4639
1. Biology Branch, OASDC, Etiology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanism of Complement Fixation and Action

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Harvey R. Colten, M.D., Herbert J. Rapp, Sc.D., Edward J. Leonard, M.D., W. Opferkuch, M.D. and G. M. Loos, Ph.D.

Cooperating Units: Molecular Separations Unit, Biology Branch, ET, NCI and Johannes Gutenberg University, West Germany

Man Years
Total: 2.0
Professional: 1.5
Other: 0.5

Project Description

Objectives:

To apply rational complement fixation tests based on the fixation and transfer of the first component of complement to the analysis of antigen-antibody reactions, in particular in the search for cancer specific antigens.

Methods Employed:

The model for studying cytotoxic reactions mediated by antibody and complement consists of sheep erythrocytes, hemolytic antibody and guinea pig complement. Purification procedures for antibodies and the complement components include: preparative (large scale) gel filtration, ion exchange chromatography and preparative free electrophoresis. Other techniques used include precipitin and immunoelectrophoretic analysis, analytical, zonal and preparative ultracentrifugation and other immuno- and physico-chemical methods.

Major Findings:

The ultimate goal of this work is the development of immunological tools that permit exploration of (1) immunological approaches to cancer diagnosis and (2) possible host immune defense mechanisms against cancer. During the last year we have explored further in depth the mechanism whereby complement components are fixed to cells in the complement fixation reaction and the distribution of active and nonactive complement components in the cytotoxic reaction. We found that under certain conditions a single antibody-antigen complex on a cell surface can fix clusters of the fourth and second (C4-C2) components of complement. As a result of this reaction, a single complement fixing antibody may generate as many as 100 cytolytic C4-C2 complexes on the cell. This reaction, therefore, represents an outstanding example of biological amplification.

We were able to isolate from serum a factor that counteracts the amplification mechanism. This factor accelerates the decay of the C4-C2 complex. The decay acceleration factor (DAF) of guinea pig serum is a heat stable (1 hr, 56°C) α-globulin, has a molecular weight of about 300,000 (estimated by gel filtration) and sedimentation rate constant of 8.2 S; DAF of human serum has a M.W. of about 170,000. DAF can be adsorbed to red cells at a rate that is independent of temperature. The activity of a given amount of DAF is independent of the number of C4-C2 complexes per cell and is dependent on the number of cells present in the reaction mixture. While DAF^{hu} accelerates the decay of C4-C2 made with $C2^{hu}$ and ${}^{oxy}C2^{hu}$ and $C2^{gp}$, DAF^{gp} is capable of accelerating the decay of guinea pig C4-C2 complexes but not those made with C2 of human origin.

Significance to Biomedical Research and the Program of the Institute:

Complement fixation is one of the most widely used diagnostic tools. The development and successful application of a radically new and very sensitive CF test, the C1 FT test, opened up new possibilities in determining antibody-antigen reactions on cell surfaces.

Furthermore, cytotoxic reactions due to antibody and complement are prime examples of body defense mechanisms. We believe that fundamental research into the nature and mechanism of complement fixation and action will contribute greatly to the development of diagnostic tools and to the understanding of the mechanism of immune body defenses.

Proposed Course of Project:

This is a long-range project, and we expect little change in the scope of the work during the next few years. The ultimate goals of these projects are the development of better diagnostic tools based on fixation of complement and the gaining of insight into the molecular events associated with the action of complement and antibodies. It is hoped that as a result of our program of inquiry into the basic problem of the interaction of antibodies, antigens and components of complement tools will be developed that are of practical significance in our search for cancer antigens.

Publications

Borsos, T., Rapp, H.J., and Colten, H.R.: Immune hemolysis and the functional properties of the second (C2) and fourth (C4) components of complement. I. Functional differences among C4 sites on cell surfaces. J. Immun. 105: 1439-1446, 1971.

Opferkuch, W., Rapp, H.J., Colten, H.R., and Borsos, T.: Immune hemolysis and the functional properties of the second (C2) and fourth (C4) components of complement. II. Clustering of effective C42 complexes at individual hemolytic sites. J. Immun. 106: 407-413, 1971.

Opferkuch, W., Rapp, H.J., Colten, H.R., and Borsos, T.: Immune hemolysis and the functional properties of the second (C2) and fourth (C4) components of complement. III. The hemolytic efficiency of human and guinea pig C2 and C4. J. Immun. 106: 927-931, 1971.

Borsos, T., and Opferkuch, W.: Kinetics and assay of complement components: Clustering and hemolytic competence of $C\overline{42}$ complexes at a single site. Symposium Proceedings of Canadian Society of Immunology, in press.

Serial No. NCI-4639

Opferkuch, W., Rapp, H.J., Colten, H.R., and Borsos, T.: The first component of complement: Hemolytic assays with EA, EAC4 and with rat and guinea pig late acting components. Immunochem., in press.

Author: Rapp, H.J., and Borsos, T.: Molecular Basis of Complement Action. New York, Appleton-Century-Crofts, 1970, 184 pp.

SUMMARY REPORT
CHEMISTRY BRANCH
July 1, 1970 - June 30, 1971

The goal of the Chemistry Branch is to develop an understanding of the molecular events underlying carcinogenic transformation by chemical, physical, and viral carcinogens, their interactions, and to clarify the role of exogenous and endogenous factors in transformation. This program includes studies on the interaction of carcinogenic agents with living organisms at the molecular, cellular, tissue, and host levels, and seeks to clarify the consequences of these interactions in terms of cell regulation and control. Cancer is clearly a disease of genetic expression where phenotype is altered in a heritable manner. This alteration of gene expression can be due to either genetic or epigenetic events and results in altered patterns of gene expression at the level of RNA transcription, translation, and metabolic control. The central focus of the Branch is to understand how carcinogens are activated or deactivated by enzymatic processes and how the active forms interact with the gene action system producing the modifications which represent transformation. The aim of our studies is to understand these processes with a view towards the modification, elimination, or prophylaxis of the carcinogenic response in the human population.

The Cell Growth Regulation Section studies the regulatory factors in cell metabolism involved in the maintenance of the normal functional state and which, when altered, result in the conversion of normal cells to malignant cells. Our studies on the biochemistry of malignant transformation of cells by RNA tumor viruses (and on reproduction of these viruses) were part of the research base for the recent discovery of specialized enzymes of RNA-dependent DNA synthesis. An intracellular viral DNA may be the "replicative form" of the virus, and this DNA would have potential for interaction with cellular genes. Introduction of an anomalous substitute for thymidine in newly infected cells resulted in mutant viral progeny, indicating that viral DNA, in fact, acts as a basic genetic form for reproduction of virus. Attempts to extract the viral DNA from infected cells continue; the physical demonstration that an intracellular viral DNA exists is necessary to establish unequivocally the phenomenon of RNA acting as a genetic primer for DNA.

An understanding of transformation of cells by RNA tumor viruses requires a knowledge of biochemical events in infected cells, and studies continue on the intracellular synthesis of viral RNA. The small amount of viral RNA synthesized in comparison to normal cell RNA thus far has prevented the identification of intracellular viral RNA. Nonetheless, the manipulation of cells to selectively promote viral RNA synthesis or depress cellular RNA synthesis has yielded results which may lead to a biochemical description of transformation and virus reproduction.

Malignant transformation of cells occurs in two stages, an initial genetic change and a subsequent phenotypic change. Transformation of cells by Rous sarcoma viruses has provided a system to analyze these stages. The requirement for DNA synthesis in transformation, established previously in this laboratory, is completed within eight hours after infection, indicating the stabilization of the viral genes within the cell. However, experiments involving manipulation of cell density demonstrate that transformation of an infected cell can be influenced by neighboring cells. Such phenotypic suppression of malignancy may be important in tumorigenesis in nature, and the biochemistry involved in this suppression is under investigation.

Other experiments analyzing the specific biochemical changes responsible for transformation of chick embryo cells by Rous sarcoma virus are in progress. A biochemical definition of the change to malignancy in this specific case would be an important step in the resolution of the nature of tumorigenesis.

The change of cells from normal to malignant is intimately related to the regulation of RNA and protein synthesis in those cells. Much attention has recently been focused on the role of tRNA in chemical carcinogenesis. Transfer RNAs can regulate protein synthesis, and examination of the tRNAs of various organs has revealed important differences in seryl-tRNA. An especially interesting finding is the presence of a type of seryl-tRNA which recognizes a codon (UGA) not used for protein synthesis in bacterial systems. A simplified procedure for isolating tRNAs which may be applied to cells with malignant potential is under development. These studies will enhance our ability to examine regulation during carcinogenesis. Other studies have revealed a process of "positive control" of genetic function, which extends the classical "negative control" type of regulation described by Jacob and Monod. Specifically regulating the metabolism of maltose through interactions of the genes, RNA, and protein, such studies are relevant to the understanding of similar processes in oncogenesis since they bring to bear new patterns of cell regulation.

The Molecular Carcinogenesis Section studies are focused on the molecular transformation of carcinogens and potential carcinogens to either their active or detoxification forms. During evolution human and other mammalian organisms have been exposed to a variety of carcinogens and other foreign compounds and have thus developed metabolic systems for their elimination. Studies in this section have shown that these detoxification reactions are influenced by a variety of environmental factors, such as drug intake, pollutant level, as well as previous exposure to carcinogens. We have found that these systems which are primarily detoxification mechanisms may also be involved in the activation of ingested procarcinogens to their ultimate carcinogenic forms. These findings represent a base for worldwide studies now being initiated on polycyclic hydrocarbon activation. The enzymes metabolizing carcinogens, particularly the polycyclic hydrocarbons, have been studied intensively in respect to the biological control mechanisms regulating their synthesis, degradation, and activity. A variety of tissues, species, and strains of mice have been examined and found to contain this enzyme system. The level and activity of the enzyme system is being examined in respect to the susceptibility of specific strains of mice to tumorigenesis by polycyclic hydrocarbons. The enzyme has also been found in fetal tissues and can be induced transplacentally.

The regulation of the synthesis of this enzyme has been hampered by an inability to free the enzyme from its multicomponent complex of microsomes. Recently, we have been successful in separating the various protein components of microsomes by electrophoretic gel techniques and have measured the synthesis of each component by the use of radioactive tracers. Specific components of the microsomal complex increase during enzyme induction while other components remain stable. In other studies we have found that the enzyme system has a relatively short half-life of 3-4 hours, and that an extremely brief exposure of the cell to the inducer, 2 minutes or less, is sufficient to produce a maximum induction for 6-7 hours.

In other studies, aimed at the modification of carcinogenesis, a large number of polycyclic hydrocarbons, flavones, and other complex derivatives of polycyclic hydrocarbons, have been examined for their inducing activity and for their ability to inhibit the enzyme system. Examination of these large numbers of compounds has yielded three compounds - one of which has a very high inducing activity, and two of which are capable of inducing the enzyme system. These are presently being examined for their effect on polycyclic hydrocarbon tumorigenesis in mouse skin.

In other studies, the metabolic profile of benzpyrene metabolites, produced by control and induced liver enzymes as well as enzymes from skin, is being examined. The differences in metabolic profile may relate to the susceptibility of specific tissues to the carcinogenic action of polycyclic hydrocarbons. These methods have been developed using radioactive benzpyrene and thin-layer chromatography to determine the metabolic profiles of over 12 different metabolites. Thus, we are seeking the reaction intermediate in polycyclic hydrocarbon tumorigenesis, and good progress has been made in the development of methodologies for these studies. Our studies have discovered the: 1) positive role of the microsomal enzymes in the binding of polycyclic hydrocarbons to nucleic acids; 2) the positive role of this enzyme complex in polycyclic hydrocarbon toxicity; 3) the discovery of a potent inhibitor of the microsomal hydroxylase system which is also capable of (a) inhibiting benzpyrene and dimethylbenzanthracene cytotoxicity, and (b) inhibiting by more than 80-90% the tumorigenic activity of dimethylbenzanthracene; and 4) the definition of the metabolic activation stage of tumor initiation to occur in the first 12 hours of exposure.

The Nucleic Acids Section studies are in progress on the mechanism of nitrosamine carcinogenesis. The role of diet has been examined and we have confirmed that a protein deficient diet protects the rat against the hepatotoxic effects of dimethylnitrosamine (DMN) while simultaneously increasing the induction of renal tumors. It is possible to produce a 100% incidence of kidney tumors in the rat with a single dose of NMN. Other studies have shown that N-methyl-N-nitrosurea (NMNU) inhibits the incorporation of labeled precursors into rat liver, kidney, and intestine DNA earlier and much more severely than the incorporation of precursors into RNA. Since a single dose of NMNU produces tumors in nearly 100% of the animals, and this dose is very rapidly metabolized, studies with this system are ideally suited for examining the early metabolic events in chemical carcinogenesis. Studies are currently underway to determine whether these potent carcinogens affect the macromolecular structure of DNA and the DNA repair mechanisms in mammalian cells.

Studies were initiated on the role of DNA repair mechanism in the etiology of cancer. These will determine: 1) whether there are correlations between the effectiveness of repair mechanisms and resistance to tumorigenesis; 2) whether chemical and physical carcinogens interfere with DNA repair; and 3) if they do, whether the mechanism of this effect is the damage inflicted on DNA directly or the damage to DNA repair enzymes or both. These studies have begun with normal human fibroblasts with cells srom patients with xeroderma pigmentosum. These studies are in the stage of technique development.

Studies are near completion on the role of molecular conformation in determining the electrophoretic properties of polynucleotides in agarose-acrylamide gels. These important studies have indicated that it will be possible, with very small amounts of biological material, to determine whether a newly isolated polynucleotide (e.g. from a new virus) is RNA or DNA, single- or double-stranded, circular or linear. Similar determinations should be possible on newly found polynucleotides isolated in the presence of large amounts of normal cellular nucleic acids. These studies will be of utmost importance in clarifying the nature of changes in RNA and DNA during tumorigenesis.

Electron microscopic and other physical studies of the RNA of RNA-containing tumor viruses have been continued in order to specify more precisely the structure of the genome of these viruses (i.e. their molecular weight and conformational properties). Evidence is accumulating that the low molecular weight RNA species found in these viruses are degradation artifacts. Further studies are planned on the interaction between these RNA and their coat proteins. Other studies, including electron microscopic autoradiography, are aimed at further elucidation of the events which follow infection of a cell with RNA tumor viruses. We have found that the (apparently) RNA-directed, DNA polymerase activity induced in these cells by viral infection is situated chiefly in the cytoplasm of the cell. It is planned to amplify these studies using metabolic inhibitors and other precursors of macromolecular synthesis. These studies are of utmost importance in understanding viral transformation and possible viral chemical carcinogen interaction.

The Protein Section is continuing the following studies:
1) RNA metabolism in HeLa cells. Using the techniques described in last year's report, the pathways of rapidly labeled RNAs in HeLa cells have been investigated with a precision and comprehensiveness not previously possible. We find two new classes of RNA of molecular weight of approximately 5 and 10 million daltons and a ribosomal RNA precursor, molecular weight 2.6 million. Our data suggest that the 32S RNA is a precursor to both 18 and 28S ribosomal RNA and that the previously postulated "45S" RNA is in fact DNA. DNA was found to have the following unexpected properties: (a) it is heavily labeled with uridine in HeLa cells; (b) it is (by more than 80%) the largest target for the methylation; (c) the uridine and methyl of DNA labeling is specifically prevented by actinomycin D; and (d) the electrophoretic properties of DNA are specifically altered by heat treatment used by others to extract RNA. It will be many months before the significance of these findings can be fully understood; they are revolutionary with respect to our understanding of the synthesis of RNA in the nucleus and may be of key importance in understanding regulation of cells during transformation.

2) The technology described in Part 1 above has been modified slightly to study RNA metabolism in mammary tissue as described last year. We have extended and confirmed our earlier results, that mammary explants cultured in the presence of hydrocortisone have a larger percentage of heavy RNA and that prolactin acts as a general stimulant for the production of RNAs, without specifically altering the type. The cells must be incubated with hydrocortisone for at least 48 hr before the heavier RNAs may be detected. Cortisone substantially reduces the degradation of RNA, which takes place normally in mammary tissue in the absence of the hormone. These studies are designed for a study of the role of unique RNA synthesis during mammary tumorigenesis.

3) Permeation sites. In attempting to identify the kind of RNA produced in the nucleus of the HeLa cell, we are devising an assay for base composition of these species of RNA. We have found that there are two major pathways for the entrance of nucleosides into the cell, one for purine and one for pyrimidine nucleosides. Antagonism between adenosine and guanosine for the purine site is vigorous, but pyrimidines apparently have no effect on this system. An analogous situation exists at the pyrimidine site. The mechanism for this control is not known. It differs from control mechanisms studied earlier by Steck and Bader in that the level of nucleosides is 100- to 1000-fold more dilute. In the course of this work we are developing methodology suitable for the simultaneous study of high polymer RNA and nucleotide pools, which we anticipate will permit a detailed examination of the flux of RNA precursors through the heavier weight RNA and into the ultimately more stable forms.

4) Haptoglobin study. Persons heterozygous at the haptoglobin locus (type 2-1) have been found to have in their serum not only their characteristic protein, but also the proteins found in both homozygous conditions. We have estimated the quantitative relationships involved and find, on the average, that the haptoglobin 2-1 individual's serum has haptoglobin as follows: 1-1, 12%; 2-1, 75%; 2-2, 13%. Haptoglobin subtyping is expected to be the subject of a contractual effort in the next year, and efforts to optimize this technique are in progress.

5) We have discovered a more slowly migrating form of DNA from mouse cells and have established that it is identical with low density "satellite DNA" previously described. The ease and accuracy with which the mouse satellite DNA may be studied by electrophoresis will permit extensive studies of the role of this form of DNA in the cellular mechanism. Especially, we are conducting studies on the binding of carcinogen to satellite DNA which are incomplete at present.

A laboratory research program is being developed around mammary carcinogenesis and the dual role of prolactin in producing normal differentiation of mammary function and serving as a "promoter" in the development of mammary cancer. Thus, we will follow changes in mammary tissue under the stimulation of prolactin in an effort to learn as many details of the biochemical transformations that lead to the production of milk proteins (normal outcome) or malignancy ("promotion action"). We are planning studies on the determination of serum prolactin. We wish to make (by contract arrangements) measurement on various age groups in various physiological conditions, with and without malignancy, to assess the significance of serum levels of this hormone in the development of breast cancer. We expect to initiate studies of prolactin binding to mammary tissue to determine the initial reaction to prolactin activation and its role in carcinogenesis.

Extensive determinations are planned on serum haptoglobin type in patients with carcinoma of the pancreas and other diseases to study the role of this protein in defining tumor-susceptible perons. This program requires developmental aspects, especially for subtyping, which will be carried out in our laboratory, and population studies, which we anticipate will be carried out by contract in other institutions. Knowledge of the existence of a specifically susceptible population would identify persons suitable for prospective study and help to define the role of the environmental agents in carcinogenesis.

The awareness of a form of DNA in the nucleus which does not contain genetic information in the classical sense provides a stimulus to the imagination. We wish to consider whether such "structural DNA" may be involved in the control of mitotic events. In addition to carcinogen binding studies, we plan other lines of investigation which may show more clearly the significance of this form of DNA to carcinogenesis.

Serial No. NCI-4748
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Synthesis and Characterization of the RNA of RNA-Containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: John P. Bader, Ph.D., Edwin J. Matthews, Ph.D., and Yoshiyuki Kitano, Ph.D.

Other Investigators: Tsuyoshi Kakefuda, M.D., Ph.D. and Artrice V. Bader, Ph.D.

Cooperating Units: Nucleic Acids Section, C.B., and Electron Microscopy Section, Viral Oncology

Man Years:	
Total:	3.7
Professional:	3.0
Other:	0.7

Project Description

Objectives:

To determine the molecular structure of the RNA of RNA-containing tumor viruses and to elucidate the intracellular site and mechanism of synthesis of viral RNA.

Methods Employed:

Cell culture, radioactive tracer techniques, density-equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, polyacrylamide gel electrophoresis, electron microscopy.

Major Findings:

The RNAs of avian leuko-sarcoma viruses and murine leukemia virus were characterized previously in this laboratory, and recent experiments have described further the state of the viral RNA within the virion. Core particles devoid of virion envelope have been isolated as well as smaller nucleoids. A unique conformation of the viral RNAs and an unusual intravirion association with protein have been found. These characteristics are being used to reveal and analyze the intracellular state of viral RNA.

Studies of the kinetics of radioactive labeling demonstrated that viral RNA appeared in completed virions within 80 minutes after intracellular synthesis. Experiments on the synthesis of viral RNA, its location, and its function now are restricted to this interval precluding complications due to contaminating extracellular virus. A variety of metabolic inhibitors are used to analyze the effects of cellular syntheses on viral RNA.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the nature of malignant transformation by RNA tumor viruses requires a determination of the intracellular activities of viral RNA. These studies are a direct approach to an understanding of biochemical mechanisms involved in carcinogenesis.

Proposed Course of Project:

To define the site and kinetics of viral RNA synthesis, to identify factors involved in viral RNA synthesis, to follow the newly synthesized molecules into cellular organelles, and ultimately to describe the products of the viral genes.

Publications

Bader, J.P., Steck, T.L., and Kakefuda, T.: The Structure of the RNA of RNA-Containing Tumor Viruses. In Arber, W., Braun, W., Cramer, F., Haas, R., Henle, W., Hofschneider, P.H., Jerne, N.K., Koldovský, P., Koprowski, H., Maaløe, O., Rott, R., Schweiger, H.G., Sela, M., Syrucek, L., Vogt, P.K., and Wecker, E. (Eds.): Current Topics in Microbiology and Immunology. Berlin/Heidelberg/New York, Springer-Verlag, 1970 (Vol. 51) pp. 105-113.

Bader, J.P., Brown, N., and Bader, A.V.: Characteristics of Cores of Avian Leuko-Sarcoma Viruses. Virology 41: 718-728, 1970.

Bader, J.P.: The RNA of RNA-Containing Tumor Viruses. In IVth Intl. Symp. on Comparative Leukemia Research (in press).

Serial No. NCI-4750
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Factors Affecting Transformation of Cells by Avian and Murine Sarcoma Viruses

Previous Serial Number: Same

Principal Investigators: Joseph M. Clark, M.D. and
John P. Bader, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.2
Other:	0.1

Project Description

Objectives:

To determine factors involved in the initiation and development of cellular transformation induced by RNA-containing tumor viruses.

Methods Employed:

Cell culture, focus forming assays of Rous sarcoma virus, radioactive tracer techniques, nucleic acid analysis, pH stabilization with non-volatile buffers, EDTA treatment.

Major Findings:

This laboratory had previously established that transformation of cells by RNA tumor viruses requires DNA synthesis during the first 8 hours after infection. In a correlated observation, cultures of high cellular density were found to be less susceptible to transformation by RNA tumor viruses.

Transformation of chick embryo cells by Rous sarcoma virus is dependent upon cell density with maximum transformation occuring at $5X10^5$ cells/6 cm plate. DNA and RNA synthesis per cell are also a function of cell density. Maximum nucleic acid synthesis and maximum susceptibility to transformation by Rous sarcoma virus occur at the same cell density. Thus, the requirement for DNA synthesis for transformation is related to the maximum DNA synthesis found under physiological conditions.

Although maximum transformation is correlated to maximum DNA synthesis, the extent of DNA synthesis and transformation with respect to cell density are not similarly correlated, implicating the involvement of another factor(s) in transformation. Similarly, studies involving replating of dense cultures after infection showed a discrepancy between the resulting effect on transformation and DNA synthesis. Furthermore, cell growth continues beyond the cell density at which maximum transformation occurs.

Replating of infected dense cultures caused an increase in morphological transformation demonstrating that genetic transformation can occur without phenotypic transformation. Ultimately transformation is affected by environmental factors such as cell density.

Morphological transformation does not free cells from the effects of cell density. Rous focus size and the size of focus cells is inversly proportional to cell density. A heavy cell overlay imposed after infection of sparse cells decreases transformation, even when added later than 8 hours after infection.

Chick embryo cells had been shown earlier to be more susceptible to infection and transformation if infected during the S phase of the cell cycle. The observed effects of cell density on transformation cannot be attributed to cell cycle variations, since the experimental cells grew in partial synchrony independently of the number of cells plated. The use of the non-volatile buffers Hepes and Tricene demonstrated that the cell density effects on transformation are not due to pH.

Significance to Biomedical Research and the Program of the Institute:

These findings demonstrate that physiological factors operate at the cellular level to affect malignant transformations, and experimental conditions have been established to investigate these factors. An understanding of these factors is relevant to the development of tumors in differentiated tissues, and eventual control of cancer in man may involve a manipulation of such factors.

Proposed Course of Project:

To further examine the factors involved in the development of the malignant state after infection by sarcoma virus is genetically established.

Serial No. NCI-4753
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Analysis of Regulation of the Maltose System in *Escherichia coli*

Previous Serial Number: Same

Principal Investigators: Maurice Hofnung, Ph.D., Maxine Schwartz, Ph.D., and Dolph Hatfield

Other Investigators: None

Cooperating Units: Pasteur Institute, Paris France

Man Years:	
Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To understand the regulation of maltose metabolism in *Escherichia coli*.

Methods Employed:

Genetic crosses with *Escherichia coli* containing mutations in various genes affecting maltose metabolism and enzyme analyses.

Major Findings:

At least three proteins are involved in maltose metabolism in *Escherichia coli*. They are the amylomaltase, the phosphorylase and the maltose permease. These proteins are located in different regions on the genetic map of *Escherichia coli*, and the regions are designated *mal* A and *mal* B. In the *mal* A region, *mal* P is the structural gene for the phosphorylase (M. Schwartz, Ann. Inst. Pasteur 673, 1967), *mal* Q is the structural gene for amylomaltase and *mal* T is the structural gene for a regulatory protein.

Complementation analysis of the mal A region shows that mal T is outside the mal P-mal Q operon and that the controlling genetic sites of the mal P-mal Q operon are between mal T and mal P. These observations provide further evidence that maltose metabolism is under positive control mediated by the mal T gene product.

The maltose permease is located in the mal B region and there are two genes which are involved in maltose permeation. Also, there is a gene (or genes) in mal B involved in λ receptor formation.

Significance to Biomedical Research and the Program of the Institute:

The classical demonstrations of "negative" regulatory mechanisms of protein synthesis have been extended to show that positive controls exist as well. These studies analyze the "positive" mechanism. Elucidation of cellular regulatory mechanisms are essential to an understanding of the malignant potential of the tumor cell.

Proposed Course of Project:

Upon completion of the genetic crosses in the mal B region (being carried out at the Pasteur Institute) this project will be terminated.

Serial No. NCI-4758
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Analysis of Ser-tRNA in Mammalian Tissues

Previous Serial Number: NCI-4752

Principal Investigators: Dolph Hatfield, Franklin Portugal, Ph.D. and Ione Polacow, Ph.D.

Other Investigators: None

Cooperating Units: Viral Carcinogenesis Branch, NCI

Man Years:

Total:	1.70
Professional:	0.95
Other:	0.75

Project Description

Objectives:

To study in detail the difference in Ser-tRNA patterns and ratios which have been observed in beef brain and liver.

Methods Employed:

tRNA is obtained from beef brain and liver and charged with labeled serine. Individual species of Ser-tRNA are separated by the reverse phase chromatographic technique of Kelmers, et al. (J. Biol. Chem. 210: 3975, 1965).

Major Findings:

An analysis of tRNA differences of beef brain and liver has been completed. The studies provide strong evidence that the elution patterns obtained from columns reflect intracellular tRNA differences.

Fractionated beef liver Ser-tRNA gave the following codon responses: Peak I, AGU and AGC; Peak II, UCG; Peak III, UCU, UCC and UCA; and Peak IV, UGA; and beef brain: Peaks I and II, AGU and AGC; Peak III, UCG; Peaks IV and V, UCU, UCC and UCA; and Peak VI, UGA. A species of Seryl-tRNA was obtained from rabbit and from chicken liver which also recognized the nonsense codon, UGA.

Significance to Biomedical Research and the Program of the Institute:

Such experiments may elucidate control mechanisms of protein biosynthesis at the level of translation and provide a tool for studying differentiation in different tissues. The phenotypic changes observed during carcinogenesis may involve alterations in the translation of messenger RNA. Understanding the mechanism of translation will lend insight to tumor cell metabolism.

Proposed Course of Project:

A continuing analysis of Ser-tRNA in mammalian tissues.

Publications

Hatfield, Dolph, and Portugal, Franklin H.: Seryl-tRNA in Mammalian Tissues: Chromatographic Differences in Brain and Liver and a Specific Response to the Codon, UGA. Proc. Natl. Acad. Sci. U.S. 67: 1200-1206, 1970.

Hatfield, Dolph, Portugal, Franklin H. and Caicuts, Mary: Transfer RNA Specificity in Mammalian Tissues and Codon Responses of Seryl Transfer RNA. Cancer Research (in press).

Serial No. NCI-4759
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Nonsense Codon Recognition in Mammalian Tissues

Previous Serial Number: None

Principal Investigators: Dolph Hatfield, Franklin Portugal, Ph.D. and Ione Polacow, Ph.D.

Other Investigators: None

Cooperating Units: Viral Carcinogenesis Branch, NCI

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description

Objectives:

To determine if species of tRNA (Suppressor tRNAs) occur in higher organisms which recognize the nonsense codons, UAG, UAA and UGA.

Methods Employed:

Labeled UAG, UAA and UGA are being prepared by the procedure of Leder, et al. (Biochem. 4: 1561, 1965). Fractionated tRNA will be assayed for nonsense codon recognition by the codon assay of Nirenberg and Leder (Science 45: 1399, 1964).

Major Findings:

In progress.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biolcgy is whether tRNA may play a role in cellular regulation and in carcinogenesis. One approach to elucidating these problems is a study of the occurrence of suppressor tRNAs.

Proposed Course of Project:

Prepare labeled codons, fractionate tRNA and perform assays.

Serial No. NCI-4765
1. Chemistry Branch, OASDC, Etiology
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the Intracellular Replicative Genome of RNA-Containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: John P. Bader, Ph.D., Yoshiyuki Kitano, Ph.D.

Other Investigators: Artrice V. Bader, Ph.D.

Cooperating Units: Electron Microscopy Section, Viral Oncology NCI-4813

Man Years:
Total: 1.7
Professional: 1.0
Other: 0.7

Project Description

Objectives:

To delineate the biochemical events which establish the replicative form of RNA-containing tumor viruses, and to identify that replicative form.

Methods Employed:

Cell culture, radioactive tracer techniques, density equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, molecular hybridization techniques, enzymological analyses, polyacrylamide gel electrophoresis.

Major Findings:

Earlier studies in this lab demonstrated a requirement for new DNA synthesis during the early stages of infection by RNA tumor viruses. This demonstration and the finding that no new protein synthesis was necessary for the synthesis of this DNA led directly to the recent discovery of RNA-dependent DNA synthesizing enzymes in RNA tumor viruses. The presence of these latter enzymes established a biochemical rationale for viral RNA acting as a genetic template for the synthesis of viral DNA.

The use of 5-bromodeoxyuridine (BUdR) provided genetic support for an intracellular viral DNA which acts as the replicative form for the virus. Lethal and sublethal mutations were found after incorporation of BUdR into DNA during early stages of infection. Noninfectious virus particles were produced containing intact but presumably defective viral RNA, and temperature-sensitive (conditional lethal) mutants were isolated from virus populations produced after exposure of cells to BUdR. The most likely interpretation of these data is that a faulty (BUdR-containing) DNA is synthesized complementary to the infecting viral RNA, that this DNA is transcribed to make progeny viral RNA, and in the process of this transcription defective viral RNA is synthesized.

Significance to Biomedical Research and the Program of the Institute:

The demonstration that RNA can act as genetic material for the synthesis of DNA is a fundamental finding in biology. This alone would be of importance to an understanding of carcinogenesis, and the fact that the phenomenon occurs in tumor viruses advertises its relevance. It is possible that the eventual prevention or cure of certain cancers will have its basis in these observations.

Proposed Course of Project:

To extract from infected cells the DNA replicative forms of RNA-containing tumor viruses. The interaction of this replicative form with cellular genes requires a biophysical demonstration of its existance.

Publications

Bader, J.P. and Bader, A.V.: Evidence for a DNA Replicative Genome for RNA-containing Tumor Viruses. Proc. Natl. Acad. Sci. U.S. 67: 843-850, 1970.

Bader, J.P. and Brown, N.R.: Induction of Mutations in an RNA Tumor Virus using a DNA Precursor Analogue. Nature (in press).

Serial No. NCI-4717
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Effect of Various Carcinogens, Drugs and Agents in the Environment on Genetic Transcription

Previous Serial Number: Same

Principal Investigators: Raphael Salomon, Ph.D. and Harry V. Gelboin, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D.

Cooperating Units: Protein Section

Man Years:
Total: 1.2
Professional: 1.2
Other: 0.0

Project Description

Objectives:

To determine the effect of polycyclic hydrocarbons on protein synthesis of the microsomal membranes during aryl hydrocarbon hydroxylase (AHH) induction.

Methods Employed:

Separation of microsomal fraction from rat liver cells and from hamster embryo cells in culture. Solubilization of microsomal proteins by detergent and phospholipase or by phenol urea acetic acid, separation of the membrane proteins by disc gel electrophoresis.

Major Findings:

The use of detergents such as sodium deoxy cholate (DOC) and sodium lauryl sulfate (SDS) did not result in complete solubilization of the membrane proteins. This was determined by the use of radioactive amino acids. The fraction of the proteins removed from the membrane by detergent treatment was still in the form of aggregates which were too large to penetrate acrylamide gels. The use of phenol urea acetic acid (PUA) as solubilization agent has the advantage of complete solubilization of membrane proteins (90% or more). For this study of determining minor protein changes which take place in AHH induction, the complete solubilization and penetration of the gel by the membrane proteins was crucial. Using those methods in combination with double labeling technique, the proteins were separated into thirteen major bands and at least two minor bands which can be grouped into three groups (1-3, 4-9, and 10-13). The second group, which is the major group of proteins, contains approximately 60% of the radioactivity incorporated into the proteins applied to the gel. A small but consistent (approximately 10%) increase of radioactive amino acid incorporation was observed in AHH induced microsomal proteins as determined on total soluble proteins and as seen from the radioactivity distribution in the gel. The third group of protein bands showed a relatively low incorporation of labeled amino acids. An approach to label specifically the induced system by the use of δ-amino levulinic acid, which is the heme precursor of the P-450, was found inefficient since hamster cells in culture do not incorporate this precursor.

Significance to Biomedical Research and the Program of the Institute:

This study shows that benz(a)anthracene induction of AHH causes an increase of amino acid incorporation into the induced microsomal proteins and thus point to the possibility of a new enzyme synthesis as a result of exposure to hydrocarbons. This relates to the role of these enzymes in human cancer.

Proposed Course of Project:

To isolate from the microsomal fraction the smallest portion which retains the AHH activity in order to study the changes taking place in the enzymatic system following the exposure to hydrocarbons.

Serial No. NCI-4760
1. Chemistry Branch, OASDC,
Etiology
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Role of Enzymatic Hydroxylation to Polycyclic Hydrocarbon Transformation and the Relation of Cell Transformation and Reversion to Enzyme Presence

Previous Serial Number: NCI-4755

Principal Investigator: Harry V. Gelboin, Ph.D.

Other Investigator: Leo Sachs, Ph.D.

Cooperating Units: Section of Genetics, Weizmann Institute of Science, Rehovot, Israel

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description

Objectives:

To study aryl hydrocarbon hydroxylase in various cell lines under different physiological conditions and the relationship between the levels of enzymes and cell transformation and reversion.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy, and radioisotope studies are used in this project.

Major Findings:

A variety of inhibitors of enzyme activity have been tested for their effect on polycyclic hydrocarbon metabolism and transformation. 7,8-benzoflavone was found to prevent both. Other inhibitors are now under test.

Serial No. NCI-4760

Significance to Biomedical Research and the Program of the Institute:

Polycyclic hydrocarbons represent major contaminants in the environment and are serious threats to the human organism in a highly industrialized society. The enzyme system dealing with these agents is aryl hydrocarbon hydroxylase, and our findings indicate that the enzyme system is responsible for the activation of the hydrocarbon to a toxic form. The enzyme system is also responsible for detoxification, but this is probably a latter step in the enzyme action.

Proposed Course of Project:

Variants from cells transformed by chemical carcinogens with a reversion of properties characteristic of transformation will be produced, and the properties of these variants will be analyzed.

The toxic effects of various metabolites of the inducers on these cells will be studied.

The effect of the metabolites on the morphologic transformation. The effect of inhibitors of enzyme synthesis or inhibitors of enzyme activity on the toxic and transforming effect of chemical carcinogens.

Serial No. NCI-4766
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cell Regulatory Controls of Microsomal Hydroxylases Metabolizing Polycyclic Hydrocarbons

Previous Serial Number: NCI-4724

Principal Investigators: Friedrich J. Wiebel, M.D., and Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.3
Other:	0.4

Project Description

Objectives:

To study the control mechanisms regulating the synthesis and degradation of the microsomal enzyme system that metabolize polycyclic hydrocarbons in a controlled environment such as tissue culture.

Methods Employed:

Cell culture techniques, fluorospectrophotometry, recording spectrophotometry, radioisotope techniques.

Major Findings:

Aryl hydrocarbon hydroxylase is highly inducible in rodent embryo cultures and several established cell lines. The enzyme system can be stabilized in tissue culture by the presence of benz(a)anthracene, an inducer and substrate of the enzyme. Benz(a)anthracene (BA) also protects the enzyme complex against heat inactivation *in vitro*. 10^{-6}M BA are sufficient to provide a 50% protection. The protective action of BA requires the presence of NADPH. Other polycyclic hydrocarbons such as

DMBA, DBA(ac) and Naphthacene, all inducers of AHH in hamster embryo cells have similar stabilizing effects.

Significance to Biomedical Research and the Program of the Institute:

The enzyme system studied is involved in the metabolism of a large number of drugs, carcinogens, and environmental pollutants as well as a variety of endogenous compounds. It is a key factor in the response of the human organism to potentially toxic and carcinogenic foreign compounds.

Proposed Course of Project:

To study the mechanisms that regulate the level of enzyme activity. To devise means to control the activity of the enzyme system and the direction of alternative metabolic pathways. To determine the role of the enzyme complex in chemical carcinogenesis *in vitro*.

Publications:

Gelboin, H. V.: Mechanisms of induction of drug metabolism enzymes. In LaDu, Jr., B. N. (Ed.): *Part 2, Drug Biotransformation; Environmental and Genetic Factors which Modify Drug Metabolism. Fundamentals of Drug Metabolism and Disposition*. Williams and Wilkins, Baltimore, Maryland (in press).

Gelboin, H. V.: Mechanisms of induction of drug metabolism enzymes. In Brodie, B. B. and Gillette, J. R. (Eds.): *Concepts in Biochemical Pharmacology: Handbook of Experimental Pharmacology*. Springer-Verlag, New York (in press).

Conney, A. H. and Gelboin, H. V.: Antagonism and potentiation of drug action and the mechanism of microsomal enzyme induction. In Meyler, L. and Peck, H. M.: *The Handbook of Drug-Induced Diseases*. Amsterdam, The Netherlands, Excerpta Medica Foundation, Chapter 10, 1970 (in press).

Serial No. NCI-4770
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: To Characterize and Quantitate the Metabolites of Benzo(a)pyrene formed In Vivo and In Vitro in Different Tissues and Species and to Search for an Active Carcinogenic Form

Previous Serial Number: Same

Principal Investigator: Nadao Kinoshita, Ph.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:
Total: 1.8
Professional: 1.0
Other: 0.8

Project Description

Objectives:

To determine quantitatively the metabolites of benzo(a)pyrene in vitro and in vivo.

Methods Employed:

Column chromatography, thin-layer chromatography, spectrophotometry, radioactive tracer technique, animal carcinogenesis experiments, enzymology.

Major Findings:

Benzo(a)pyrene tumorigenesis is not effected or stimulated by 7,8-benzoflavone whereas 7,12-dimethylbenz(a)anthracene is markedly inhibited. 7,8-benzoflavone inhibits aryl hydrocarbon hydroxylase activity in rat liver microsome induced with methylcholanthrene, but stimulates the enzyme in control microsomes. Both control and induced mouse skin enzyme is inhibited by 7,8-benzoflavone. A slightly stronger inhibition is observed with the induced enzyme. These differences of action of 7,8-benzoflavone between control and induced enzymes show that there are two kinds of enzyme systems involved in the hydroxylation of aryl hydrocarbons. We are finding different metabolic patterns of benzo(a)pyrene formed by induced and control enzymes. We are trying to identify the metabolite of benzo(a) pyrene which is closely related with benzo(a)pyrene carcinogenesis.

Significance to Biomedical Research and the Program of the Institute:

The active form of polycyclic hydrocarbons is not known. Benzo(a)pyrene is one of the powerful carcinogens of the polycyclic hydrocarbon class which exists in our environment as a pollutant. This study firstly will show the metabolites of benzo(a)pyrene which relate closely with carcinogenesis and further studies concerning characterization of active forms which may be a key to understanding polycyclic hydrocarbon carcinogenesis. This may enable us to alter the course of chemical carcinogenesis in the human.

Proposed Course of Project:

To further examine the carcinogenesis and characterization of metabolites of 7,12-dimethylbenz(a)anthracene which bring the opposite result to benzo(a)pyrene concerning effect of 7,8-benzoflavone on mouse skin tumorigenesis.

Serial No. NCI-4778
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Microsomal Aryl Hydrocarbon Hydroxylases: Different Forms and their Role in Toxicity and Carcinogenicity

Previous Serial Number: NCI-4767

Principal Investigators: Harry V. Gelboin, Ph.D., Friedrich J. Wiebel, M.D., and Janet Leutz

Other Investigators: Nadao Kinoshita, Ph.D. and David Williams

Cooperating Units: None

Man Years:

Total:	2.2
Professional:	1.9
Other:	0.3

Project Description

Objectives:

Microsomal hydroxylases are key factors in the response of the organism to drugs, environmental pollutants, and carcinogens. To study the different forms of the enzymes metabolizing polycyclic hydrocarbons in tissues of mammals and in tissue culture. To investigate their mode of action and to develop means to control their activity. To study the relationship between the activity of microsomal hydroxylating enzymes and chemical carcinogenesis.

Major Findings:

Aryl hydrocarbon hydroxylase (AHH) can be differentially stimulated or inhibited by 7,8-benzoflavone in microsomes from male rats. The kinetics of inhibition was of the mixed typed. The two hepatic forms of AHH vary in their proportions during development of rats and between sexes. With benzo(a)pyrene as substrate, the enzyme system was differentially inhibited by a number of carcinogenic and noncarcinogenic polycyclic hydrocarbons. Although the potent carcinogen was also a strong inhibitor, no correlation between inhibitory activity and carcinogenicity was observed. A differential effect of 7,8-benzoflavone on the microsomal enzyme complex was observed *in vivo* as well: 7,8-benzoflavone given 15 min prior to zoxazolamine did not change the paralysis time in untreated and phenobarbital pretreated rats; however, it doubled the paralysis time in rats pretreated with methylcholanthrene. This demonstrates that two forms of AHH operate *in vivo* and they can be differentially affected by 7,8-benzoflavone. Aryl hydrocarbon hydroxylase activity *in vitro* was generally measured by hydroxylation of benzo(a)pyrene. 3-hydroxy-benzo(a)pyrene, a major metabolite of the enzymatic reaction, was found to be further metabolized *in vitro* by the enzyme complex from hepatic microsomes. This reaction was similar to the hydroxylation of benzo(a)pyrene to nonhydroxy derivatives in its requirement for NADPH and Mg^{++} and its induction and inhibition characteristics. In contrast, the metabolism of 3-hydroxybenzo(a)pyrene in tissue culture was not inducible by polycyclic hydrocarbon, occurred to the same extent in cell types containing widely differing AHH activities, and was insensitive to inhibitors of AHH in tissue culture.

Significance to Biomedical Research and the Program of the Institute:

The enzyme system studied is largely responsible for the metabolism of polycyclic hydrocarbons as well as a large variety of exogenous and endogenous substances. The oxidative metabolism of polycyclic hydrocarbons frequently leads to less toxic and carcinogenic products. However, during the sequence of metabolic steps, intermediates can be formed that are highly reactive and possibly initiate the carcinogenic process. Thus, detailed knowledge is required about the level of enzyme activity in tissues and organs, the existence and distribution of different forms of the enzymes, their specific metabolic products in order to understand the process of chemical carcinogenesis, and other pharmacotoxicological effects. The use of specific inhibitors or stimulators of the enzyme system provides an important tool, to evaluate the role of the enzyme system in toxicity and carcinogenicity. It may ultimately contribute to control the response of the human body to exogenous, potentially harmful chemicals.

Proposed Course of Project:

To study the different forms and reactions of microsomal hydroxylases and their dependency on endogenous and exogenous factors. To evaluate the role of the enzyme complex in chemical carcinogenesis and to find substances that specifically control the level of enzyme activity and thus might provide protection against chemical carcinogenesis.

Honors and Awards:

Invited Lecture, "Studies on the mechanism of aryl hydrocarbon hydroxylase induction *in vivo* and *in vitro* and its role in toxicity and tumorigenicity", New York Academy of Sciences, New York, New York, July 1970.

Claude Bernard Award and Visiting Professor, "Studies on the mechanism of microsomal hydroxylase induction and its role in carcinogen action", University of Montreal, Canada, September 1970.

New Horizons Award and Lecture, "Carcinogenesis, the environment and gene action", Radiological Society of North America, Chicago, Ill., December 1970.

Symposium Speaker, "Microsomal hydroxylases: Studies on the mechanism of induction and their role in polycyclic hydrocarbon action", M.D. Anderson Hospital, Houston, Texas, March 1971.

Publication:

Gelboin, H.V. and Wiebel, F.J.: Studies on the mechanism of aryl hydrocarbon hydroxylase induction and its role in cytotoxicity and tumorigenicity. Annals N.Y. Acad. Sci. (in press).

Serial No. NCI-4779
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Evaluation of Various Compounds for their Effects as Inducers and Inhibitors of Aryl Hydrocarbon Hydroxylase Activity and Tumorigenesis

Previous Serial Number: NCI-4768

Principal Investigator: Harry V. Gelboin, Ph.D.

Other Investigators: Friedrich Wiebel, M.D., Nadao Kinoshita, N. P. Buu-Hoi, and L. Diamond

Cooperating Units: Institut du Radium, Paris, and the Wistar Institute, Philadelphia, Pennsylvania

Man Years:

Total:	2.1
Professional:	1.8
Other:	0.3

Project Description

Objectives:

To develop methods to determine the activity of various compounds as inducers or inhibitors of carcinogen hydroxylation enzymes and polycyclic hydrocarbon tumorigenesis.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy and radioisotope studies and mouse skin tumorigenesis.

Major Findings:

Over twenty-five compounds have been tested for the enzyme inducing activity in cell culture, the enzyme inhibitory activity in isolated microsomes and in cell culture and their effect on mouse skin tumorigenesis. Three of the compounds were found to have a powerful inducing effect and several inhibited the enzyme. They are now under test in tumorigenesis studies.

Significance to Biomedical Research and the Program of the Institute:

Compounds that inhibit or induce the hydroxylating enzyme systems may be possibly used to alter the course of tumorigenesis.

Proposed Course of Project:

To reveal a variety of compounds that can regulate enzyme activity and polycyclic hydrocarbon tumorigenesis.

Publications:

Gelboin, H. V.: Chemical Carcinogens and the theories of their action. In Enciclopedia Medica Italiana, Rome, Italy (Ed. 2) (in press).

Gelboin, H. V., Wiebel, F. J., and Diamond, L.: Dimethylbenzanthracene tumorigenesis and aryl hydrocarbon hydroxylase in mouse skin: Inhibition by 7,8-benzoflavone. Science 170: 169-171, 1970.

Williams, D., Leutz, J., Wiebel, F.J., and Gelboin, H.V.: The effect of polycyclic hydrocarbons in vitro on aryl hydrocarbon (benzo(a)pyrene) hydroxylase. Biochem. Pharmacol. (in press).

Wiebel, F.J., Leutz, J., Diamond, L. and Gelboin, H.V.: Aryl hydrocarbon (benzo(a)pyrene) hydroxylase in microsomes from rat tissues: Differential inhibition and stimulation by benzoflavones and organic solvents. Arch. Biochem. Biophys. (in press).

Serial No. NCI-4780
1. Chemistry Branch, OASDC, Etiology
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Inhibitory Effect of 7,8-Benzoflavone on Mouse Skin Tumorigenesis by 7,12-Dimethylbenz(a) anthracene

Previous Serial Number: None

Principal Investigators: Nadao Kinoshita, Ph.D. and Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.8
Professional:	0.8
Other:	2.0

Project Description

Objectives:

To examine the role of aryl hydrocarbon hydroxylase for *in vivo* binding of 7,12-dimethylbenz(a)anthracene to biomacromolecules with the powerful inhibitor of the enzyme, 7,8-benzoflavone, and investigate the correlation between 7,12-dimethylbenz(a)anthracene carcinogenesis and its metabolism.

Methods Employed:

Animal carcinogenesis experiments, spectrophotometry, radioactive tracer techniques, enzymology.

Major Findings:

7,8-benzoflavone does not inhibit the induction of aryl hydrocarbon hydroxylase on skin by topical application of 7,12-dimethylbenz(a)anthracene, but markedly inhibits enzyme activity when added *in vitro*. 7,12-dimethylbenz(a)anthracene forms covalent linkage to the DNA, RNA and proteins of mouse skin. 7,8-Benzoflavone inhibits by 70, 60 and 50% respectively. 7,8-Benzoflavone inhibits 7,12-dimethylbenz(a)anthracene initiated tumor formation by 80-90% when applied simultaneously with 7,12-dimethylbenz(a)anthracene. Maximum inhibition of tumor formation appears when both compounds were applied simultaneously and no effect was observed when 7,8-benzoflavone was applied more than 12 hours before or after 7,12-dimethylbenz(a) anthracene.

Significance to Biomedical Research and the Program of the Institute:

The studies suggest that carcinogen may be converted to an active form by the enzyme and the part of the tumor initiation process by carcinogen is completed in less than 12 hours. An understanding of the nature of these phenomena may offer potential for the prevention or alteration of normal cell to malignant cell conversion.

Proposed Course of Project:

To determine the active form of 7,12-dimethylbenz(a)anthracene in carcinogenesis; to study which binding of carcinogen to biomacromolecules is related to normal cell to malignant cell conversion.

Serial No. NCI-4751
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acid Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electron microscopy of RNA from RNA-Containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: Tsuyoshi Kakefuda, M.D., Ph.D., and John P. Bader, Ph.D.

Other Investigators: Masakazu Hatanaka, M.D., Ph.D., and Emily A.O. Callan

Cooperating Unit: Flow Laboratories, Rockville, Maryland

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description

Objectives:

To determine the structure of the ribonucleic acid of RNA-containing tumor viruses by direct viewing in the electron microscope.

Methods Employed:

High molecular weight RNAs from murine leukosis-sarcoma virus, Avian leukosis-sarcoma virus, mammary tumor virus, rat leukosis virus and viper leukosis virus were isolated by SDS-phenol extraction. These were spread with DEAE-dextran on a surface of distilled water, transferred onto a supporting film on a grid, washed and air dried. The specimens were then either shadowed with platinum-carbon or stained with uranyl acetate for visualization in the electron microscope. In other experiments the viral RNAs were treated with agents which dissociate inter- and intra-molecular hydrogen bonds for the study of any conformational changes which might occur.

Major Findings:

All of the viruses studied contained two major components; high (50 to 70S) and low (~5S) molecular weight RNAs, as detected by zone sedimentation in sucrose density gradients, polyacrylamide gel electrophoresis, and electron microscopy. The high molecular weight RNAs were between 8 and 14μ long, the average length of which varied depending upon the strain of the virus used. Murine sarcoma virus RNA from virus grown in a rat cell line were in collapsed forms initially which then became extended and linear after treatment with urea and the same length (14μ) as the RNA from MSV grown in a mouse cell line. Other RNAs were of linear form without the urea treatment. The relative number of short to medium sized molecules increased considerably after fractionating the total RNA in a sucrose gradient, indicating degradation of the molecules occurs during this procedure. The majority, if not all, of the low molecular weight RNAs were, therefore, considered degradation products rather than a native component of the viral genome.

Significance to Biomedical Research and the Program of the Institute:

The structure of the genomes of RNA-tumor viruses has been analyzed extensively by biochemical and biophysical methods. However, the conversion of a sedimentation coefficient to molecular weight depends upon knowing the conformation of the RNA under investigation. Direct observation by electron microscopy provides useful information on the molecular organization of the genome of these viruses.

Proposed Course of Project:

It is planned to extend the comparative study on the structures of these RNAs to a variety of tumor viruses which are different in biological (carcinogenic) and antigenic properties. Interaction between the RNA and coat protein necessary for virus assembly will also be studied biochemically and electron microscopically.

Serial No. NCI-4784
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Role of Molecular Conformation in Determining the Electrophoretic Properties of Polynucleotides in Agarose-Acrylamide Gels

Previous Serial Number: None

Principal Investigator: C. Wesley Dingman, M.D.

Other Investigators: M. Patricia Fisher, Tsuyoshi Kakefuda, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	0.8
Other:	0.7

Project Description

Objectives

To determine whether polyacrylamide gel electrophoresis under controlled conditions would be a useful technique for defining the structure of newly isolated or discovered polynucleotides.

Methods Employed:

A number of different polynucleotides of known structures were isolated from viral, bacterial, and mammalian sources. Their electrophoretic behavior in composite agarose-acrylamide gels was studied under different conditions of temperature, gel concentration, and voltage gradient.

Major Findings:

Above a molecular weight of 0.3 to 0.4×10^6 double-stranded (ribo- or deoxyribo-) polynucleotides showed an increase in mobility with an increase in temperature and an increase in mobility with an increase in voltage gradient, unlike single-stranded ribopolynucleotides. Double-stranded species also showed unusually low retardation coefficients for their size (the retardation coefficient, K_R, is defined thusly: Log mobility = log mobility at zero gel concentration - K_R multiplied by the gel concentration). These results led us to hypothesize that double-stranded polynucleotides migrate "end-on" in these gels. We also showed that linear double-stranded polynucleotides of different molecular weights are resolvable when their weights range from about 0.3×10^6 (or possibly lower) to 2×10^6. In the molecular weight range of 1 to 3×10^6, circular single-stranded molecules may be resolved from linear single-stranded molecules having the same molecular weight, and circular double-stranded polynucleotides may be resolved from linear double-stranded molecules having the same molecular weight. In this same weight range, "nicked" open circular double-stranded DNA may be separated from intact supercoiled circular double-stranded DNA. These attributes of polynucleotides, together with their different sensitivities to different endonucleases and their different affinities for certain dyes, could enable one to determine in many cases the nature of a previously uncharacterized polynucleotide (i.e., whether it is RNA or DNA, whether it is single- or double-stranded, and whether it is circular or linear).

Significance to Biomedical Research and the Program of the Institute:

The results of this project will enable many investigators in both virology and nucleic acid metabolism to ascertain, rather inexpensively, the major structural aspects of newly isolated polynucleotides of biomedical significance. Furthermore, the techniques developed under this project are particularly useful when the amount of available material for investigation is very small. Thus, it is hoped that these procedures will aid in defining the nature of the genome of newly isolated viruses of oncogenic significance as well as allow for the possible description of the alterations which oncogenic viral genomes undergo from infection to integration with the host genome.

Proposed Course of Project:

To publish these results and then to explore their applicability to the problem of defining the nature of the events which occur involving the invading and host genomes following infection of mammalian cells with oncogenic viruses.

Publications

Fisher, M.P., and Dingman, C.W.: The Role of Molecular Conformation in Determining the Electrophoretic Properties of Polynucleotides in Agarose-Acrylamide Gels. Biochemistry (in press).

Serial No. NCI-4785
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Role of DNA Repair Mechanisms in the Etiology of Cancer

Previous Serial Number: None

Principal Investigators: Richard J. Brown, M.D., C. Wesley Dingman, M.D.

Other Investigators: Peter G. Burk, M.D., Peter F. Swann, Ph.D., and M. Patricia Fisher

Cooperating Units: Dermatology Branch, OASDCR, General Labs and Clinics, NCI

Man Years:
Total: 1.4
Professional: 1.2
Other: 0.2

Project Description

Objectives:

To learn more regarding the role of DNA repair mechanisms in maintaining the integrity of the mammalian genome. In particular, to determine (a) whether there are correlations between the effectiveness of repair mechanisms and the resistance to tumorigenesis of different tissues, (b) whether chemical and physical carcinogens interfere with DNA repair, and (c) if they do, whether the mechanism of this effect is the damage inflicted on DNA directly or the damage to DNA repair enzymes or both.

Methods Employed:

We have started this project by attempting to find (if it exists) a difference in the effectiveness of DNA repair in normal human fibroblasts and lymphocytes and those from patients with xeroderma pigmentosum. (Evidence collected by others indicating that the first step in DNA repair of ultraviolet light induced damage is deficient in these patients is not entirely convincing.) Cells are grown in tissue culture, DNA is labeled with various appropriate radioactive precursor molecules, the cells are irradiated with ultraviolet light, and the DNA isolated at different times thereafter. Evidence for the excision of the induced defect and the repair of the excised region is looked for. *In vitro* enzymatic assays may be employed at a later date if feasible. Similar studies are planned using carcinogenic alkylating agents administered *in vivo* to susceptible and non-susceptible tissues as the means for introducing defects in DNA.

Major Findings:

This project has just recently been started and no major findings of significance have been forthcoming as yet.

Significance to Biomedical Research and the Program of the Institute:

Only within the last few years has the problem of DNA repair and its potential importance for confering resistance to mutagenesis (and possibly carcinogenesis) received much attention. It would appear that understanding these systems and their role in the overall economy of the cell would benefit many areas of biomedical research (especially those pertaining to genetic dysfunction) and aid in understanding the etiology of at least some forms of cancer (e.g., skin cancer).

Proposed Course of Project:

To pursue the goals outlined above in *Objectives*.

Serial No. NCI-4786
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Intestinal Cancer: The reaction between 1,2-dimethylhydrazine and cellular constituents of the rat and mouse *in vivo*

Previous Serial Number: None

Principal Investigators: Peter F. Swann, Ph.D.

Other Investigators: Andrew Hawks, M.D., Peter N. Magee, M.B., F.R.C. Path.

Cooperating Units: Isotope Laboratory, Building 20, NIH, and Courtauld Institute, Middlesex Hospital Medical School, London, England

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description

Objectives:

Intestinal cancer is one of the most common human cancers. The experimental approach to this problem was revolutionized when it was discovered that tumors of the colon could be specifically and selectively induced in rats and mice with 1,2-dimethylhydrazine. The object of this project was to synthesize 1,2-dimethylhydrazine with the methyl groups labeled with ^{14}C and to examine the metabolic fate of this compound in the animals and to determine whether there was any reaction between the compound and the nucleic acids of the cells of the colon.

Methods Employed:

1,2 [^{14}C] dimethylhydrazine was synthesized from hydrazine by the following steps:

$$^{14}CH_3I + AgOSO_2CH_3 \rightarrow {}^{14}CH_3OSO_2CH_3$$

$$(NH_2)_2 + HCOOH \rightarrow (NH.CHO)_2$$

$$(\mathrm{NH.CHO})_2 + 2\,{}^{14}\mathrm{CH_3OSO_2CH_3} \rightarrow (\mathrm{N.CHO.}{}^{14}\mathrm{CH_3})_2$$

$$(\mathrm{NCHO.CH_3})_2 \xrightarrow{\mathrm{HCl}} \mathrm{CH_3NH.NHCH_3}$$

Preliminary experiments to follow the fate of this compound in the mouse have been carried out by Dr. Andrew Hawks and Professor P.N. Magee in England.

Major Findings:

Preliminary experiments suggest that this compound reacts as an alkylating agent in a manner similar to the carcinogenic N-nitroso compounds. The amount of alkylation of N-7 of guanine in mouse colon *in vivo* was similar to that produced in the rat by a single dose of N-methyl-N-nitroso urea, a compound which also induces colon cancer.

Significance to Biomedical Research and the Program of the Institute:

These experiments strengthen the view that a number of different compounds produce cancer by a common chemical mechanism. These studies should aid in the discovery of this mechanism and the reason for the peculiar susceptibility of the colon.

Proposed Course of Project:

To be continued when Dr. Swann returns to England at the end of his Fellowship.

Serial No. NCI-4787
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mode of Replication of RNA Tumor Viruses

Previous Serial Number: None

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: Emily A.O. Callan

Cooperating Units: None

Man Years:

Total	1.0
Professional:	0.5
Other:	0.5

Project Description

Objectives:

To observe the sequential cellular events in the course of oncogenic viral RNA replication.

Methods Employed:

Tissue culture cells were infected with an RNA tumor virus and then incubated with ^{3}H-thymidine. After pulse or pulse-chase labeling for different times the cells were fixed, acid washed and prepared for light and electron microscopic autoradiography. Other cells were either not infected or infected with UV-inactivated virus for controls.

Major Findings:

Confluent 3T3 cells infected with murine sarcoma virus and incubated with ^{3}H-thymidine for more than 6 hours showed a large number of grains in the cytoplasm. Prolonged incubation caused a heavy labeling in both the nucleus and cytoplasm. At early stages after infection cells showed labeling only on the surface membrane suggesting that DNA synthesis for viral replication occurs preferentially in this region. Non-infected cells and cells infected with UV-inactivated virus had no appreciable number of grains in the cytoplasm.

Significance to Biomedical Research and the Program of the Institute:

Evidence is being accumulated for the concept that the RNA of RNA tumor viruses is replicated through the transient intermediate synthesis of a specific DNA. The enzyme necessary for this synthesis has been found in RNA tumor viruses as well as in human leukemia cells. Although it is still questionable that RNA-dependent DNA replication occurs *in vivo*, our findings favor this possibility.

Proposed Course of Project:

In this study we plan to observe the more advanced stages of viral RNA replication in susceptible cells. Inhibition of viral replication by drugs and concomitant cytological changes will also be studied.

Serial No. NCI-4788
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Nitrosamine Carcinogenesis: The effect of nitroso compounds on the function and fate of nucleic acids

Previous Serial Number: None

Principal Investigator: Peter F. Swann, Ph.D.

Other Investigators: C. Wesley Dingman, M.D. and M. Patricia Fisher

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.7
Other:	0.2

Project Description

Objectives:

Carcinogenic N-nitroso compounds seem to have a common characteristic: During their metabolism they each produce an alkylating intermediate which reacts with constituents of the cell including nucleic acids. The role of this alkylation of nucleic acids in the carcinogenic process is unknown nor is it clear how this alkylation affects the function and fate of nucleic acids. Recently methods, particularly gel electrophoresis, have been developed which may be capable of demonstrating the maturation, the transport, and the decay of the major classes of RNA. One N-nitroso compound, N-methyl-N-nitroso urea will produce cancer in many tissues of the rat when given as a single dose. This dose is completely decomposed within 15 minutes of injection. Thus, this compound seemed ideally suited for a study of the effect of a carcinogen on nucleic acid structure and metabolism, the objective of this project.

Methods Employed:

Incorporation of precursors into RNA and DNA in various tissues of the rat is measured at intervals after injection of N-methyl-

N-nitroso urea, and analysis of the newly synthesized RNA in nucleus and cytoplasm is carried out by means of gel electrophoresis. Analysis of the macromolecular structure of both RNA and DNA following these treatments is carried out by means of both gel electrophoresis and zone centrifugation in sucrose gradients.

Major Findings:

This work is still in a very preliminary stage. It has been shown, however, that the incorporation of precursors into DNA is affected earlier and much more severely than incorporation of precursors into RNA. Preliminary results also indicate that these agents may lead to fragmentation of DNA *in vivo*.

Significance to Biomedical Research and the Program of the Institute:

A single dose of N-methyl-N-nitroso urea decomposes in the rat within 15 minutes of injection yet this dose is sufficient to produce cancer in almost every one of the treated rats. If we understood the events occuring in this 15 minutes and the metabolic consequences of these events, we would have advanced a long way toward understanding cancer.

Proposed Course of Project:

To continue this study so as to accomplish as much of the objectives as possible.

Serial No. NCI-4789
1. Chemistry Branch, OASDC, Etiology
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Nitrosamine Carcinogenesis: The effect of a protein deficient diet on the toxic and carcinogenic activity of dimethylnitrosamine

Previous Serial Number: None

Principal Investigator: Peter F. Swann, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description

Objectives:

A short period of feeding a protein deficient diet protects rats against the toxic action of dimethylnitrosamine. A single dose of dimethylnitrosamine given to rats on this diet produces a much higher incidence of kidney tumors than a similar dose given to rats on a normal commercial rat food. The object of this project was to complete experiments began in England to explain this.

Methods Employed:

Slice preparations were made in the conventional manner, from the livers of rats on a normal and protein deficient diet and the ability of each to metabolize dimethylnitrosamine was measured by polarographic assay of the amount of nitrosamine remaining in the flask after incubation. The effect of graded doses of dimethylnitrosamine given to rats on the normal and protein deficient rat, on the histological appearance of the liver is also being measured.

Serial No. NCI-4789

Major Findings:

It has been shown that the ability of the rat liver but not of the kidney, to metabolize dimethylnitrosamine is decreased by a protein deficient diet. As a result, more of the dose is metabolized in the kidney, and the incidence of tumors produced in the kidney greatly increased.

Significance to Biomedical Research and the Program of the Institute:

This study gives a method of producing a 100% incidence of kidney tumors with a single dose of carcinogen. It reemphasizes the importance of diet and the induction of drug metabolism enzymes in carcinogenesis.

Proposed Course of Project:

To publish these results.

Serial No. NCI-4718
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Disc Electrophoresis - A Study of the Fractionation of Protein Mixtures on Cylindrical Columns of Polyacrylamide Gels

Previous Serial Number: Same

Principal Investigator: Arthur T. Ness, Ph.D.

Other Investigators: Jullia V. Pastewka and Andrew C. Peacock, Ph.D.

Cooperating Units: None

Man Years:
Total: 2.1
Professional: 2.1
Other: 0.0

Project Description

Objectives:

There are five objectives: (a) study of the variables involved in the fractionation of protein mixtures; (b) identification of the fractions and their biochemical significance; (c) determine and interpret the significance of the changes produced by systems known to alter the secondary and tertiary structures of proteins; (d) study of protein-protein interactions and related variables; (e) study of the effect of hormones and other factors on the induction and rate of biosynthesis of proteins by various cells and tissues.

Methods Employed:

1. The disc polyacrylamide gel electrophoresis technique as developed by Ornstein and Davis and as modified by others for particular systems.

2. The use of other fractionation techniques such as column chromatography, preparative gel electrophoresis, ultracentrifugation, etc., to obtain homogeneous, unaltered protein entities to aid in the identification of the components obtained in disc

gel electrophoresis and in the interpretation of the results obtained in studies outlined in the broad objectives.

3. The technique of isoelectric focusing in disc acrylamide gel systems. This establishes a pre-selected pH gradient range (wide or narrow) and permits the separation of proteins of isoelectric point difference of 0.02 pH unit.

Major Findings:

1. The studies of the structure of haptoglobins and of the unusual reaction between haptoglobins and hemoglobins were continued.

Present evidence indicates that the Hp 1-1 molecule is made up of α^1 and β subunits, the Hp 2-2 molecule of α^2 and β subunits, and the Hp 2-1 molecule of α^1, α^2, and β subunits. The subunits are bound together principally through disulfide bonds, which may be split by reductive cleavage procedures to yield the separated α^1, α^2 and β subunits which can then be resolved by disc electrophoresis on polyacrylamide gels. Haptoglobin 1-1 consists of a single molecular species, while Hp 2-1 and Hp 2-2 consist of multiple molecular species of increasing size comprised of unknown assemblages of α^1, α^2 and β subunits (Hp 2-1) and of α^2 and β subunits (Hp 2-2). The polymeric species in each case are not integer multiples of a basic unit.

Purified Hp 2-1 was electrophoresed on polyacrylamide gels, and those polymeric bands, where the resolution permitted, were individually excised from the gels and then the protein was extracted from pooled excised material for the individual protein. The extracts were electrophoresed to insure homogeneity. Portions were then reductively cleaved and electrophoresed. Stained gels were scanned to quantitate the amounts of α^1 and α^2 proteins. This data should give some clue to the assemblage of the subunits of some of the bands of Hp 2-1. The diffuseness of the α^1 band made quantitation uncertain, so further studies of the electrophoretic systems are being made to effect sharper bands and thus increase the reliability of quantitation measurements.

2. Persons heterozygous at the haptoglobin locus (type 2-1) have been found to have in their serum not only their characteristic protein, but also the proteins found in both homozygous conditions. We have estimated the quantitative relationships involved and find, on the average, that the haptoglobin 2-1 individual's serum has haptoglobin as follows: 1-1, 12%; 2-1, 75%; 2-2, 13%. Haptoglobin subtyping is expected to be the subject of a contractual effort in the next year, and efforts to optimize this technique are in progress.

3. Restudied and improved the disc polyacrylamide gel procedure for the haptoglobin subtyping of serums.

Significance to Biomedical Research and the Program of the Institute:

The high resolution and sensitivity of the disc electrophoretic technique permit study of the occurrence and significance of genetic variants of protein entities and also the analysis of subcellular fractions to determine the site or sites of protein synthesis and a study of the biochemical factors in protein synthesis. The technique may also permit the detection and assessment of physiological changes associated with the development of malignancy.

Proposed Course of Project:

1. Continue the study of the isolated polymeric proteins of Hp 2-1 and Hp 2-2 to determine the subunit assemblages of these proteins.

2. Use the disc gel technique to measure the appearance of proteins in mammary tissue to evaluate changes in mammary tissue resulting from exposure to carcinogenic agents.

Publications

Pastewka, J.V., Ness, A.T., and Peacock, A.C.: Alterations in polyacrylamide gel disc electrophoretic patterns of hemoglobin after exposure to fluorescent light. Anal. Biochem. 35: 160-166, 1970.

Peacock, A.C., Pastewka, J.V., Reed, R.A., and Ness, A.T.: Haptoglobin-hemoglobin interaction. Stoichiometry. Biochemistry 9: 2275-2279, 1970.

Serial No. NCI-4721
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Electrophoretic Techniques

Previous Serial Number: Same

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigator: Roswell A. Reed

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.3
Other:	0.0

Project Description

Objectives:

To study electrophoretic properties of nucleotides. To develop analytical procedures for the estimation of nucleotides in samples suitable for RNA analysis and to estimate the base composition of RNA species isolated only by gel electrophoresis.

Methods Employed:

Standard electrophoretic techniques with the use of chromatographic and ultracentrifugal methods to obtain samples suitable for study. Radioisotope counting of ^{14}C and tritium. Optical scanning of stained and unstained gels. Tissue culture of malignant cells.

Major Findings:

In attempting to identify the kind of RNA produced in the nucleus of the HeLa cell, we are devising an assay for base composition of these species of RNA. We have found that there are two major pathways for the entrance of nucleosides into the cell, one for purine and one for pyrimidine nucleosides. Antagonism between adenosine and guanosine for the purine site is vigorous, but pyrimidines apparently have no affect on this system. An analogous situation exists at the pyrimidine site.

The mechanism for this control is not known. It differs from control mechanisms studied earlier by Steck and Bader in that the level of nucleosides is 100- to 1000-fold more dilute. In the course of this work we are developing methodology suitable for the simultaneous study of high polymer RNA and nucleotide pools, which we anticipate will permit a detailed examination of the flux of RNA precursors through the heavier weight RNA and into the ultimately more stable forms.

Significance to Biomedical Research and the Program of the Institute:

The role of very heavy ribonucleic acids characteristically found in rapidly dividing tissues has been speculated to be either (a) messenger RNA or (b) regulatory RNA. Characterization of these RNA species in normal and malignant tissues may show light on differences in the control mechanisms in malignant cells.

Proposed Course of Project:

Techniques developed thusfar through the use of HeLa cells will be extended and applied to measurements made in mammary tissue. Estimates will be made of possible differences in base composition influenced by hormonal composition of the medium. We will study the influence of the mammary tumor virus on the production of this RNA, and in particular, we will try to use the base composition to help identify the presence of mammary tumor virus in suitably infected tissues.

Publications

Dahlberg, A.E., and Peacock, A.C.: Studies of 16S and 23S ribosomal RNA of *Escherichia coli* using composite gel electrophoresis. *J. Mol. Biol*. 55: 61-71, 1971.

Serial No. NCI-4731
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrophoretic Analysis of RNA

Previous Serial Number: Same

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigator: Sylvia L. Bunting

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.3
Other:	0.0

Project Description

Objectives:

To devise methods for the extraction of RNA and DNA from malignant cells in a manner best fitting to preserve the native state and to provide samples suitable for electrophoretic analysis.

Methods Employed:

Cultures of HeLa cells, assay of radioactivity, rapid isolation of RNA, and electrophoretic analysis of RNA.

Major Findings:

Using the techniques described in last year's report, the pathways of rapidly labeled RNAs in HeLa cells have been investigated with a precision and comprehensiveness not previously possible. We find two new classes of RNA of molecular weight of approximately 5 and 10 million daltons and a ribosomal RNA precursor, molecular weight 2.6 million. Our data suggest that the 32S RNA is a precursor to both 18 and 28S ribosomal RNA and that the previously postulated "45S" RNA is in fact DNA. DNA was found to have the following unexpected properties: (a) it is heavily labeled with uridine in HeLa cells; (b) it is (by more than 80%) the largest target for the methylation; (c) the uridine and methyl of DNA labeling is specifically prevented by actinomycin D; and (d) the electrophoretic properties of DNA are specifically altered by heat treatment used by others to extract RNA. It will be many months before the significance of these findings can be fully understood; they are revolutionary with respect to our understanding of the synthesis of RNA in the nucleus.

Significance to Biomedical Research and the Program of the Institute:

The rapidly labeled RNAs of high molecular weight that occur in the nucleus of a cell occupy a central role in its physiology. These findings provide a new insight into the relationships that exist between these species. In particular, most of the species previously thought to be ribosomal may now be considered to play either a regulatory or an informational role. A clarification and resolution of the issues presented by this report will provide a much deeper insight into the metabolism of the cell.

Proposed Course of Project:

We hope to clarify whether the RNAs are informational or regulatory, and we wish to study the process by which the cell controls the transition from one form to another. Since the conversion of ribosomal RNA from its precursors is correlated with cell growth, we plan to determine whether the conversion is a cause or a result of increased cellular proliferation.

Serial No. NCI-4781
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Electrophoretic Characteristics of Mouse Satellite DNA

Previous Serial Number: None

Principal Investigator: Robert S. Zeiger, M.D., Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D., and Raphael Salomon, Ph.D.

Cooperating Unit: Molecular Carcinogenesis Section

Man Years:
Total: 1.7
Professional: 1.7
Other: 0.0

Project Description

Objectives:

Study the electrophoretic characteristics, carcinogenic binding, and methylation of mouse DNA using agarose-acrylamide composite gels.

Methods Employed:

Mouse DNA is analyzed by electrophoresis in composite agarose-acrylamide gels and by cesium chloride density gradient centrifugation. Isotopic experiments include labeling of DNA with ^{3}H-(methyl)thymidine, ^{3}H-(methyl)methionine, and ^{3}H-dimethylbenz(a)anthracene (DMBA).

Major Findings:

Deoxyribonucleic acid (DNA) extracted from mice either by the phenol or Marmur methods was separated electrophoretically into two bands on composite polyacrylamide-agarose gels. On a 2.5% polyacrylamide-0.5% agarose slab gel a major fast-moving (FMB) and minor slow-moving (SMB) band were observed in both newborn and adult mice but not in guinea pig, rat, or man. The SMB migrated 0.5 mm/hr slower than the FMB at 200V and comprised 9%

of the total cellular DNA. Both bands were degraded by DNAase but not RNAase and incorporated methyl-^{3}H-thymidine proportionally to their concentrations. To characterize these electrophoretic bands, mouse DNA was separated by cesium chloride (CsCl) density gradient centrifugation into major (high density) and satellite (low density) fractions. The low density DNA migrated on electrophoresis mainly (85%) with the SMB; the high density DNA migrated entirely with the FMB. After electrophoretic separation on preparative gels of DNA labeled in newborn mice with methyl-^{3}H-thymidine, the SMB was eluted electrophoretically from the gels combined with unlabeled high density DNA and subjected to CsCl centrifugation. Most of the radioactivity peaked at 1.691, the density of satellite DNA. On the other hand, the FMB band similarly eluted banded at a density of 1.701, corresponding to main band DNA. These results indicate that the SMB observed on gel electrophoresis of mouse DNA is composed of low density DNA and is equivalent to mouse satellite DNA obtained by CsCl centrifugation.

Methylation of DNA was investigated utilizing secondary mouse embryo cultures. These cultures were grown for 40 hr in methionine-deficient media, 2 mg/ml sodium formate, and 200 μc ^{3}H-(methyl)methionine per plate. DNA was extracted by a rapid phenol-SDS method and electrophoresed on composite gels as described above. There was greater than a twofold increase in incorporation of the methionine in the SMB (satellite DNA) than the FMB (main DNA) relative to DNA concentration as determined by spectrophotometric scanning of stained gels, confirming similar results obtained by CsCl centrifugation. As such, the technique proves to be a reliable and rapid method for ascertaining differences in specific activities of these two species of DNA.

DNA was extracted from scraped epidermis 24 hr after the application of ^{3}H-DMBA to the shaved dorsal surface of mouse skin. Its specific activity was 1044 DPM/μg DNA. Electrophoresis of DMBA-bound DNA on composite gels revealed similar relative specific activities between the SMB and FMB. CsCl centrifugation showed that both satellite and main DNA had similar specific activities (950-1000 DPM/μg DNA) and therefore were bound by DMBA to the same extent.

Significance to Biomedical Research and the Program of the Institute:

The electrophoretic technique provides a rapid method for the separation of mouse satellite DNA and a reliable assay for ascertaining differences in metabolic activity of these two species of DNA in normal and malignant tissues.

Proposed Course of Project:

To ascertain structural property of DNA that determines its migratory behavior on gels, and to study the possible role of this form of DNA in carcinogenesis.

Serial No. NCI-4782
1. Chemistry Branch, OASDC, Etiology
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hormonal Effects on Normal and Malignant Breast Tissue in Culture

Previous Serial Number: None

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D., Sylvia L. Bunting, and Yale J. Topper, Ph.D.

Cooperating Unit: Section on Intermediary Metabolism, Laboratory of Biochemistry and Metabolism, NIAMD

Man Years:
Total: 1.2
Professional: 1.2
Other: 0.0

Project Description

Objectives:

To study the response of normal and malignant breast tissue in culture to hormones in terms of RNA and DNA synthesis and the production of proteins (particularly milk proteins), and to evaluate the role of prolactin in carcinogenesis.

Methods Employed:

Culture of mammary explants, isolation of RNA and DNA, electrophoretic analysis of RNA, isolation of milk proteins, assay of radioactivity, microscopic examination of explants, autoradiography.

Major Findings:

RNAs produced in explants of mouse mammary glands maintained on an insulin (I) or insulin plus hydrocortisone (IF) containing medium in the presence or absence of prolactin (P) have been studied. Electrophoretic analysis of the RNAs obtained by a very rapid isolation procedure, demonstrated that addition of P for 24 hours to explants incubated previously on I or IF for 4 days produced no significant alteration in the distribution of radioactivity in RNA following 2- or 4-hour incubations with tritiated uridine. The addition of prolactin also resulted in a doubling of the rate at which labeled precursor was incorporated into RNA of an IF system. The RNAs isolated from explants which had been on insulin medium containing hydrocortisone, whether prolactin was present or not, showed a greater percentage of slowly migrating RNA species having molecular weights of approximately 10 million, following incubation with tritiated uridine than those which had been maintained on I or IP medium.

IF explants maintained for several days in the presence of P accumulate secretory products in the lumina of the alveoli. The amount of proteins accumulated in the presence of P may be assessed by gel electrophoresis.

Significance to Biomedical Research and the Program of the Institute:

Hydrocortisone has been shown to cause an increase of enzyme or protein synthesis of several different cells or organs in culture. The mechanism by which this is brought about is unknown. We have demonstrated, perhaps for the first time, an effect of hydrocortisone on the distribution of the kinds of RNAs of mammary explants in culture. In mouse explants during short-term incubations milk proteins are produced in insulin and prolactin containing media only in the presence of hydrocoritsone even though the epithelial cells are competent to produce their specific proteins. The control of growth and differentiative function by hormones of normal and malignant tissues is of concern for our understanding of basic biological problems and an eventual understanding of the process of malignancy. The role of hormones in the exacerbation and remission of breast tumors is evident. We may hope to learn more about etiology, therapy, and possible prognosis of breast cancer from a variety of studies in which the effects of hormones on the breast tissue are considered.

Proposed Course of Project:

Because mammary tissue in culture responds to prolactin in terms of increased RNA synthesis and milk protein production, this system may be used as a more sensitive assay than is currently available for measuring amounts of prolactin present in blood. Blood from persons under extended hormone therapy for contraception and of patients who have had breast surgery for malignancy whose treatment included further surgery such as ovariectomy, adrenalectomy or hypophysectomy would be assayed for prolactin levels (under contract). Biopsy material of breast tissue from malignant tumor-bearing hosts (animal or human) may be grown and examined for responsiveness to hormones for retrospective and prognostic purposes. The effect of individual hormones on such tissue, whether the malignancy is initiated by viruses or chemicals may be determined in culture.

One of the interesting actions of hydrocortisone is its effect in increasing the amount of rough endoplasmic reticulum of mammary epithelial cells as seen under the electron microscope. Another effect described for this hormone is its membrane stabilizing ability. We plan to investigate further the role of hydrocortisone which permits prolactin to increase the amounts of RNA and milk proteins synthesized by mammary epithelial cells.

Publications

Green, M.R., Bunting, S.L., Topper, Y.J., and Peacock, A.C.: Changes in labeling pattern of RNA from mammary tissue as a result of hormone treatment. Biochemistry, in press.

Summary Report
EXPERIMENTAL PATHOLOGY BRANCH
July 1, 1970 through June 30, 1971

The Experimental Pathology Branch is the youngest Branch within the Carcinogenesis Area. The present year is its first full year of operation. The members of this Branch conduct research in their in-house laboratories but are also heavily involved in the planning and management of the contract program. Out of fifty contracts which have been in existence in the Carcinogenesis Area during the past year, the project officers of twenty and and the assistant project officers of three others have been members of the Experimental Pathology Branch. In addition, members of this Branch have served as advisors to the Food and Drug Administration and other government agencies.

The Experimental Pathology Branch plans, develops and implements a coordinated research program involving the following: (1) development and characterization of animal models for carcinogenesis research; (2) development and standardization of animal bioassay systems for carcinogenesis tests; (3) development of standards of pathology for the evaluation of carcinogenic effects; (4) correlation of chemical and toxicological data in the selection and screening of chemical carcinogens; (5) biochemical studies on the metabolism of chemical carcinogens in selected animal systems; and (6) implementation of carcinogen screening and bioassay programs using standardized methods.

The Branch is comprised of the following subdivisions:

A. Office of the Chief

(1) Histopathology Unit: (a) Plans, organizes, and conducts investigations on the pathology of processes required for the development of the carcinogenic response to chemical carcinogens using optical and electron microscopic methods; (b) develops and monitors standards of pathology for carcinogenesis studies; (c) provides assistance and advice to staff of the Carcinogenesis Area on problems relating to histopathology and ultrastructure of tumors; and (d) provides histology service for the Carcinogenesis Area.

B. Bioassay Section: (1) Plans, organizes, and conducts research to develop and select new and improved methods and techniques for the bioassay of carcinogenic agents; (2) defines and standardizes bioassay systems to obtain quantitation of carcinogenic responses; (3) defines conditions of species and organ susceptibility, age, sex, and genetic factors affecting the specificity of carcinogenic agents and the ways in which these factors influence the interaction of carcinogens with target sites.

C. Carcinogen Screening Section: (1) Develops and monitors screening programs for the detection of carcinogenic hazards of environmental chemical agents; (2) investigates the toxic, pharmacologic, and carcinogenic activity of selected chemicals or mixtures of environmental significance; and (3) explores metabolic pathways of selected test chemicals in different animal species.

As resources become available in the future, the staff of the Experimental Pathology Branch will assume a more clearly defined program function of developing animal models of the major types of human cancer and of using these models to determine what factors may be involved in the development of these types of cancer in man. The rationale for this approach to carcinogenesis is that there does not appear to be a 1:1 relationship between exposure to a chemical or viral carcinogen and the ultimate development of cancer. Secondary factors interact to enhance or inhibit the tumorigenic response to the carcinogen. In some human situations it may not be possible to prevent exposure to the carcinogen, and thus a more effective means of prevention may involve the manipulation of these secondary factors. Steps have been taken to begin implementing this program to the extent that it is feasible with current resources. Members of the Carcinogen Screening Section are beginning to plan and conduct research on cancer of the colon and rectum. One member of the Bioassay Section is assuming responsibility for research on childhood cancers and the effect of exposure of children and fetuses to carcinogens on the development of cancer later in life. Future plans call for conversion of the Histology Unit to a program for developing animal models of cancer of the endocrine target organs. Other groups should be established to work on cancer of the pancreas, urinary bladder and kidney if resources permit.

Most of this program will be conducted through contract-supported research. However, in order to effectively coordinate this research it will be essential to have a "critical mass" of staff scientists who are thoroughly immersed in the type of research which they will manage, so that they will be aware of its problems, opportunities, and developments, and can effectively serve the function of examining the plans and research of contract-supported organizations to be sure that these represent the best interests of the government.

Major advances obtained in the intramural research of the Branch are summarized in the following paragraphs:

The Histopathology Unit is concerned with the induction of carcinomas in animals given a variety of chemicals. Emphasis is placed on the changes occurring prior to the development of carcinomas, particularly at the ultrastructural level. Other work is involved with the importance of age, strain of rat, and hormones on these lesions.

Carcinomas are usually found in livers that have cirrhosis. With most chemical carcinogens younger male rats are more susceptible to cirrhosis than older males or than females. Opposite results were found with carbon tetrachloride-induced carcinomas and cirrhosis of the liver. Older animals develop more of these lesions and females are more susceptible than males.

Methylcholanthrene increases the carcinomas and nodules and cirrhosis of the liver in animals given carbon tetrachloride. The cirrhosis is increased in animals regardless of age or sex. The increase in hyperplastic nodules and carcinomas is greater in older animals and in females. The incidence of hepatic vein thrombosis and of cholangic fibers, a premalignant lesion, is higher in older rats. These lesions are increased when methylcholanthrene is given simultaneously with carbon tetrachloride, and the increase is most noticeable in older animals. Azathioprene, an immunosuppresant drug, increases the severity of cirrhosis of the liver in adult animals given carbon tetrachloride. The increase in hepatic vein thrombosis and cholangiofibrosis observed with methylcholanthrene was not observed with azathioprine.

Methylcholanthrene induces thyroiditis and carcinomas, but only in Buffalo strain rats. Younger animals are more susceptible than older, and females more than males. Temporal studies of the thyroiditis show that the lesion develops suddenly rather than over a period of time. Trypan blue also produces thyroiditis, and the lesion develops in younger animals rather than in aged animals--a finding comparable to that in animals given methylcholanthrene.

Another study was concerned with the differentiation between hyperplastic and neoplastic lesions of the liver in mice. Carcinomas can be easily transplanted, whereas hyperplastic lesions will not survive on transplantation. The findings are similar to those in rats.

An additional project was concerned with the relationship of the histologic pattern of hepatic carcinomas and metastases. It was found that undifferentiated carcinomas do not metastasize because the host animal dies from the rapid growth of the carcinoma. If the carcinoma is excised surgically, the host lives for a longer period of time and then metastases can be observed.

The Carcinogen Screening Section has continued its studies on the activation of carcinogenic aromatic amines. The first step in the activation of these compounds as well as of certain carcinogenic azo dyes is N-hydroxylation. This step, an activation process, is distinct from other hydroxylation reactions, often thought to be detoxification reactions. Selective inhibition of the activation reaction has been shown to inhibit the carcinogenic process in the liver. These findings have also been extended to other tissues and species under a variety of conditions. The second activation step, conjugation with sulfuric acid takes place inside susceptible cells. Modification of this process was secured by competitive noncarcinogenic analogous products which tie up the sulfate required for the activation reaction of the carcinogen. It has also been found that the commercial antioxidant BHT, (butylated hydroxytoluene) also inhibits the carcinogenicity to the liver of a carcinogenic aromatic amine though other antioxidants such as DPPD (diphenyl-p-phenylenediamine) or excess ascorbic acid failed to affect the carcinogenic process. Studies on the metabolism of a carcinogenic aromatic amine and a noncarcinogenic analog showed that the urinary and circulating metabolites as well as the interaction of these chemicals with cellular and molecular elements differed depending upon whether the chemicals were administered by a single intraperitoneal dose or by continuous feeding. These results demonstrate the importance of performing biochemical and pharmacologic studies with drugs under conditions simulating their actual use.

A system for the long-term culture of normal epithelial hepatic cells which was developed in this laboratory is being used to study chemical carcinogenesis. Several different types of chemical carcinogens lead to alterations in morphology, growth pattern and karyotypes of these cells relatively rapidly. However, injection of these altered cells into newborn syngeneic hosts has thus far failed to give detectable tumors. Additional tests involving irradiated animals are underway. In contrast, fibroblasts from liver of rodents undergo a spontaneous change _in vitro_ which led to sarcoma formation quickly under the same conditions.

A new and expanding part of the program of this section deals with the mechanism of induction of cancer of the colon. Preliminary results show that the toxicity and perhaps the carcinogenicity of a colon carcinogen in rats is quite susceptible to dietary modification using diets patterned after those of humans in the western world.

Research in-house and in collaborating laboratories has demonstrated the carcinogenicity or cocarcinogenicity of the following agents: 1. Drugs. 1-(4-N-Nitroso-N-methylaminobenzylidene)indene; 4-(4-N-Nitroso-N-methylaminostyryl)quinoline; Melphalan; chlorambucil; uracil mustard; Natulan; dimethyltriazinoimidazole carboxamide; BCNU; CCNU. 2. Industrial chemicals and dyestuff intermediates: _o_-toluidine; 4-chloro-_o_-toluidine; 2,5-dimethoxy-4'-aminostilbene; propylenimine; propane sultone. 3. Pesticide degradation product: Ethylene thiourea. 4. Natural environmental products, molds and toxins: Candida parapsilosis and Candida albicans; mycotoxins related to aflatoxin; cyclopropenoid fatty acids.

Several of these agents have induced brain tumors (gliomas). These tumors have been difficult to induce in the past. Thus, they may aid in the development of an appropriate animal model for studying this type of human tumor.

Another significant advance at a contract laboratory was the discovery of a new metabolite of aflatoxin, and the development of a method for its analysis. This metabolite, an O-demethyl derivative, is excreted in the urine. Thus, it may be possible to determine human consumption of aflatoxin by measuring excretion of the metabolite and thereby relating the eventual occurrence of disease to the consumption of aflatoxin. Other new analytic methods will improve the measurement of carcinogenic nitrosamines in the environment.

The Bioassay Section's in-house research is devoted to the development and utilization of biologic model systems for studying the early metabolic events of chemical carcinogenesis and to developing and exploring experimental animal models of the kinds of cancer most commonly found in children. A technique for the cell culture of fetal mouse skin developed in this laboratory is now being used for investigations on chemical carcinogenesis. Under normal culture conditions, these cells survive for a few weeks and then die out. However, when exposed to 7,12-dimethylbenz(a)anthracene (DMBA) in the solvent Tween 80, they recover their ability to proliferate and survive indefinately with an epithelial morphology. These cultures have the electron microscopic characteristics of epidermal cells and form tumors on transplantation which also have epidermal characteristics by electron microscopy. Treatment with the solvent, Tween 80, alone also results in permanent cultures which form tumors on transplantation, though the time required for recovery of cell proliferation and the time for development of transplantability are longer than in the cultures treated with DMBA.

Mice exposed in utero to the carcinogen ethylnitrosourea (ENU) are more susceptible than adults to the development of pulmonary carcinogenesis. This difference is accentuated in strains which are genetically resistant to lung carcinogenesis. Two transplantable pulmonary tumors from C3HfB mice, a strain relatively resistant to pulmonary carcinogenesis, were established from animals treated in utero with ENU. These lines grow poorly or not at all in the strain of origin but are readily transplantable to an F_1 hybrid between the strain of origin and the pulmonary tumor sensitive strain, A. However, the tumor lines grow well in animals of the parental strain whose immune mechanism is suppressed with antilymphocyte serum. Furthermore, a secondary response can be demonstrated in the F_1 hybrids by the technique of tumor implantation, subsequent excision, and challenge. These studies suggest that resistance to pulmonary tumorigenesis in mice may have an immunological basis.

Lymphocytic leukemia induced in NZW or Swiss mice by ENU sometimes undergoes regression with the concomitant development of aplastic anemia. During the past year it has been demonstrated that the lymphoproliferative condition which undergoes regression is unequivocally neoplastic, since lymph nodes excised from animals during the florid phase of the disease can be transplanted into newborn mice in which they produce a progressive, fatal, lymphocytic leukemia. Transplantable tumor lines have also been established from animals which showed histological evidence of an incomplete form of tumor regression.

Experiments on the ability of soluble polymers to induce sarcomas at the site of injection have demonstrated that the tumorigenic response is dependent on the charge of the polymer. Positively charged polymers induce sarcomas while uncharged or negatively charged polymers were ineffective. Pretreatment with ethylnitrosourea by another route of administration prior to injection of the polymer increased the incidence of sarcomas at the site of polymer injection.

Serial No. NCI-4684
1. Experimental Pathology Branch, OASDC, Etiology
2. Histopathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemical Thyroiditis

Previous Serial Number: Same

Principal Investigator: Melvin D. Reuber, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
- Total: 0.60
- Professional: 0.30
- Other: 0.30

Project Description

Objectives:

Investigate host factors involved in chemical thyroiditis in rats.

Methods Employed:

Methylcholanthrene (0.033%) was administered in the diet for 12 weeks. A 1% solution of trypan blue was injected subcutaneously. The dosage was 50 mg/kg of body weight once weekly for five weeks.

Major Findings:

(a) Buffalo Strain male and female rats, given methylcholanthrene in the diet, were killed at varying periods of time. The incidence of thyroiditis tended to increase with continued ingestion of the chemical. The lesion was observed earlier in female than in male rats, and the incidence increased faster with time on the diet. The most striking finding was the severe thyroiditis (as compared with mild or moderate) in animals, regardless of when they are killed.

(b) Female rats given trypan blue were more susceptible than male rats. Female rats, 8, 12, and 24 weeks old developed thyroiditis more often than males of the same ages or females 4 or 52 weeks old. Males, 4 and 24 weeks of age, developed a higher incidence of thyroiditis than males of other ages.

(c) Buffalo rats 76 weeks of age given methylcholanthrene in the diet were less susceptible to the development of thyroiditis than were rats 8 weeks of age.

Significance to Biomedical Research and the Program of the Institute:

Experimental thyroiditis in experimental animals has been an auto-immune disease. There has been no evidence to indicate that this chemically-induced thyroiditis in Buffalo-strain rats is autoimmune.

This lesion, with severe hyperplasia of the epithelial cells of the acini, appears to be preneoplastic. Under certain conditions carcinomas of the thyroid gland develop.

Proposed Course of Project:

The Principal Investigator accepted an academic position in March 1971. He will bring the on-going studies to an orderly conclusion and publication.

Publications

Reuber, M. D.: 3-Methylcholanthrene-Induced Thyroiditis in Buffalo Strain Rats. *Arch. Environ. Health* 21: 734-739, 1970.

Reuber, M. D.: Influence of Age and Sex on Chronic Thyroiditis in Rats given Subcutaneous Injection of Trypan Blue. *Toxic. Appl. Pharmacol.* 17: 60-66, 1970.

Reuber, M. D.: Decreased Incidence of Thyroiditis in Buffalo Strain Rats given 3-Methylcholanthrene. *Path. Microbiol.* 36: 223-229, 1970.

Serial No. NCI-4685
1. Experimental Pathology Branch, OASDC, Etiology
2. Histopathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Influence of Age and Sex on a Variety of Hepatic Lesions

Previous Serial Number: Same

Principal Investigator: Melvin D. Reuber, M.D.

Other Investigators: Kurt Stromberg, E. Lee Glover, and Sigmund Grollman

Cooperating Units: GL&C, NCI; Montgomery College, Takoma Park, Maryland; and University of Maryland, College Park, Maryland

Man Years:
Total: 0.60
Professional: 0.30
Other: 0.30

Project Description

Objectives:

Determine the influence of age and sex on the development of cirrhosis, hyperplasia and carcinomas of the liver.

Methods Employed:

Buffalo strain male and female rats 4, 8, 12, 24, 52, and 76 weeks of age were used. Cirrhosis of the liver was induced by carbon tetrachloride. Methylcholanthrene and azathioprine were given with the CCl_4. Hepatic carcinomas were induced by N-4-(4'-fluorobiphenyl)acetamide in the diet.

Major Findings:

Animals given CCl_4 only had mild or moderate cirrhosis. Those given methylcholanthrene or azathioprine in the diet simultaneously with CCl_4 developed severe cirrhosis. Methylcholanthrene also increased hyperplastic nodules and small carcinomas. The pro-cirrhotic and pro-carcinogenesis effects of methylcholanthrene were not influenced by the age of the animals.

Males given N-4-(4'-fluorobiphenyl)acetamide developed more hepatic carcinomas than female. Younger animals of both sexes had more hepatic lesions than older animals. The 4-week-old animals were particularly susceptible to the carcinogen. In addition to a higher incidence, they also had pulmonary metastases and a shorter survival time.

Significance to Biomedical Research and the Program of the Institute:

Few studies in chemical carcinogenesis have been concerned with age of animals. These experiments have systematically used rats 4, 8, 12, 24, 52 and 76 weeks of age.

Proposed Course of Project:

The Principal Investigator accepted an academic position in March 1971. He will bring the on-going stuties to an orderly conclusion and publication.

Publications

Reuber, M.D., Grollman, S. and Glover, E.L.: Severe Cirrhosis in Rats of Varying Ages Given Methylcholanthrene and Carbon Tetrachloride. *Arch. Path.* 89: 531-536, 1970.

Reuber, M.D.: Effect of 3-Methylcholanthrene on Hyperplastic and Early Neoplastic Hepatic Lesions Induced in Rats by Carbon Tetrachloride. *J. Nat. Cancer Inst.* 45: 1237-1242, 1970.

Reuber, M.D.: Accentuation of Carbon Tetrachloride-Induced Cirrhoris by Azathioprine in the Rat. *Arch. Path.* 90: 567-571, 1970.

Stromberg, K., and Reuber M.D.: Influence of Age and Sex on Hepatic Lesions Induced by Chemical Carcinogens: Ingestion of N-4(4'-fluorobiphenyl) acetamide by Buffalo Strain rats. *J. Nat. Cancer Inst.* 44: 1044-1054, 1970.

Serial No. NCI-4689
1. Experimental Pathology Branch, OASDC, Etiology
2. Histopathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Enzyme Activities in Transplantable Mouse and Rat Hepato-cellular Carcinomas

Previous Serial Number: None

Principal Investigator: Melvin D. Reuber, M.D.

Other Investigators: F. Schapira, V. R. Potter, S. Fiala, and E. Essner

Cooperating Units: Institut de Pathologie Moleculaire, Paris, France; University of Wisconsin, Madison, Wisconsin; University of Southern California, Los Angeles, California; Sloan-Kettering Institute for Cancer Research, New York, New York

Man Years:
Total: 0.35
Professional: 0.20
Other: 0.15

Project Description

Objectives:

Determine activities of several enzymes in a number of transplantable hepatomas in the rat and mouse maintained in this laboratory.

Methods Employed:

(a) Aldolose A, B, and C were studied in transplantable hepato-cellular carcinomas 178, 122, 175, and 189 in rats. (b) Tyrosine aminotransferase, serine dehydrase, glucose-6-phosphate dehydrogenase, and ATP: citrate oxaloacetate-lyase were determined for transplantable mouse hepatomas 1, 15, 2, 3, and 4. (c) Rat Hepatoma 139 was used for assay of glutathionase activity. (d) The intracellular localization of acid phosphatase reaction product was studied in transplantable hepatoma 146 in the rat.

Major Findings:

(a) Aldolose A which is relatively abundant in fetal liver was increased in slow-growing rat hepatomas. In addition to aldolose A, aldolose C, hybridized with A, was present in the fast-growing hepatomas. Aldolose C was present in fetal liver but was absent in normal adult liver, as shown by kinetic, electrophoretic and immunologic studies.

(b) Tyrosine aminotransferase activity was higher in host livers than in the hepatomas. This enzyme in hepatomas 1, 2, 3, and 5 responded to feeding a high protein diet to the host, but in hepatomas 1 and 15, the enzyme activity was not increased after feeding a low protein diet. Serine dehydratose activity was present in substantial levels in host liver, but was absent in all hepatomas in mice fed a high protein diet. Glucose-6-phosphate dehydrogenase and citrate cleavage enzyme activities were present in all host livers and hepatomas but with less diversity in their activity levels compared to those of tyrosine aminotransferase and serine dehydratose.

(c) The activity of glutathionase in hepatoma 139 was high and differed strikingly from that of normal liver.

(d) Light microscopy showed acid phosphatase staining in lysosomer and rod-shaped structures in hepatoma 146. Reaction product was localized at the electron microscope level to various structures in and near the Golgi apparatus. These included dense bodies, double-walled circular profiles, and microbodies.

Significance to Biomedical Research and the Program of the Institute:

Studies concerned with biochemical and ultrastructural changes in carcinomas will ultimately add to our understanding of the changes occurring during carcinogenesis.

Proposed Course of Project:

The Principal Investigator accepted an academic position in March 1971. He will bring the on-going studies to an orderly conclusion and publication.

Publications

Fiala, S., and Reuber, M. D.: High Glutathionase Activity in "Minimal Deviation" Reuber Hepatoma 139. *Gann* 61: 275-278, 1970.

Schapira, F., Reuber, M. D., and Hatzfeld, A.: Resurgence of Two Fetal-Type of Aldolose (A and C) in Some Fast-Growing Hepatomas. *Biochem. Biophys. Res. Comm.* 40: 321-327, 1970.

Essner, E., and Reuber, M. D.: Localization of Acid Phosphatase Activity in Well-Differentiated Hepatocellular Carcinoma 146. *J. Nat. Cancer Inst.* (In Press).

Reynolds, R. D., Potter, V. R., Pitot, H. C., and Reuber, M. D.: Survey of Some Enzyme Patterns in Transplantable Reuber Mouse Hepatomas. *Cancer Res*. (In Press).

Serial No. NCI-4611
1. Experimental Pathology Branch, OASDC, Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Metabolic Events in the Early Stages of Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Richard R. Bates, M.D.

Other Investigators: Henry Hennings, Ph.D.; Stuart H. Yuspa, M.D.

Cooperating Units: Dermatology Branch, NCI

Man Years:

Total:	5.0
Professional:	0.8
Other:	4.2

Project Description

Objectives:

The purpose of this project is to determine the importance of specific metabolic activities in neoplastic transformation by chemical carcinogens and to develop new systems of chemical carcinogenesis which are particularly suitable for this type of investigation.

Methods Employed:

The principal tissue studied has been mouse skin. Studies in the intact mouse are designed to test the effect of various drugs and hormones on the induction of skin tumors by chemical carcinogens. During the past year heavy emphasis has been placed on experiments using cell culture of fetal mouse skin. This utilizes a culture technique developed in this laboratory which permits the growth of epidermal cells in culture and their subsequent transplantation to the exposed panniculus carnosus of the mouse in such a way that they redevelop into skin on this site. Recent efforts have been devoted to finding a second type of cell suitable for this project. The most likely candidate is the mammary epithelial cell. Like the epidermal cell, this can also be cultured for short periods of time and then transplanted to its normal site in an animal where it will develop normal structures. Current activities are devoted to improving the techniques for culturing this type of cell.

The cell cultures are used for relatively long-term studies on malignant transformation induced by treatment of the cultures with chemical carcinogens. They are also used for short-term studies on the interaction of chemical carcinogens with cellular constituents and the effect of these interactions on cell function. Particular emphasis is placed on studying the interaction of carcinogens with DNA and the effect of this interaction on DNA function. Chromatographic techniques and ultracentrifugation are used to separate different types of DNA.

Major Findings:

A number of improvements have been made in the technique for culturing mouse epidermis. It has been found for example that these cells grow well in roller bottles, allowing the production of larger numbers of cells with the expenditure of less effort than was required when these cells were grown in Petri dishes. Most studies to date have been done with cultures consisting of mixed populations of epidermal cells and fibroblasts. It is possible to separate these cell types through the use of a discontinuous Ficoll gradient. With mammary gland cells, it is possible to enhance the yield epithelium and inhibit the growth of fiberblasts by culturing the cells on a collagen substrate.

Under normal culture conditions fetal mouse skin grows vigorously for several days but then gradually loses its ability to replicate and the cultures die out after a few weeks. In contrast, it was found that cultures treated with either Tween 80 or 7,12-dimethylbenz(a)anthracene (DMBA) recovered their replicative capacity, and formed monolayers of epithelial cells which ultrastructurally show the presence of desmosomes and tonofilaments. These cultures continue to proliferate indefinately. The recovery of the cultures to a mitotically active state occurs more quickly after treatment with DMBA than it does after treatment with Tween 80. Eventually both types of cultures form tumors upon transplantation to animals. Again, the ability to form tumors occurred earlier in cultures treated with DMBA than in cultures treated with Tween 80. The tumors which have developed so far have a spindle-cell morphology which is not diagnostic of any particular cell type by light microscopy. However, by electron microscopy, the cells of the tumors show the presence of desmosomes and tonofilaments and other characteristics of epidermis while lacking any of the electron microscopic characteristics of fiberblasts. Cell cultures established from these tumors also have an epidermal growth pattern with desmosomes and tonofilaments. It thus appears that poorly differentiated epidermoid carcinomas have developed from mouse skin cells treated in vitro with DMBA or with Tween 80. Since untreated cultures do not survive to the time at which malignant transformation occurs, it is not possible to determine whether the treatment of the cells directly induces the malignant transformation or whether it simply allows survival of the cells to a time at which "spontaneous transformation" occurs.

Experiments reported in the 1970 Annual Progress Report demonstrated that if mouse skin cells were incubated for a period of time with tritiated DMBA and then exposed to 5-bromodeoxyuridine (BUDR), DNA to which the DMBA had bound was capable of replicating; and in the newly synthesized DNA, most of the carcinogen was bound to the parent strand with relatively little to the daughter strand. The presence of any significant amount of DMBA in the daughter strand required explanation, however. Further experiments along these lines were conducted by incubating in tritiated DMBA, then for a number of hours in normal medium, followed by incubation in BUDR. This allowed a period of time to wash remaining unbound DMBA out of the cells and to allow those strands of DNA which were undergoing synthesis at the time the carcinogen was removed from the medium to complete their synthesis before bromodeoxyuridine was added. Under these conditions, when BUDR containing DNA was isolated, all of the hydrocarbon was found to be bound to the parent strand while there was no detectable binding to the daughter strand. Thus these experiments reinforce the previous experiments indicating that the binding of DMBA to DNA does not prevent replication, that the DMBA binds mostly to a single strand of DNA with little if any cross-linking, and that the DMBA remains bound to the DNA during the process of replication. Current investigations are designed to determine whether the presence of the DMBA on a replicating molecule of DNA has any effect on the structure of that molecule of DNA or on its subsequent function.

Significance to Biomedical Research and the Program of the Institute:

The culture and transplantation techniques for skin have become very useful tools in this laboratory for the study of the early events in malignant transformation by chemical carcinogens. It is anticipated that the development of the mammary gland culture system to the point at which it can be used in a similar way, will allow a comparison of the response to chemical carcinogens of cells in different physiologic states and thus to gain additional knowledge on the process of chemical carcinogenesis.

Since the malignant transformation is transmitted from one cell generation to the next, it is important to study cellular molecules and organelles which carry phenotypic information from one cell generation to the next. DNA is a most important example of such a molecule. Thus it is essential that we learn more of how the structure and function of this molecule is affected by chemical carcinogens.

Proposed Course of Project:

Research in future years will continue along the same general lines as those discussed here.

Honors and Awards

Member, Ad Hoc Committee on Toxicological Procedure, Office of the Commissioner, FDA.

Member, Group on Program Recommendations for National Center for Toxicological Research.

Member, Group on Facility Recommendations, National Center for Toxicological Research.

Member, Chemical Carcinogenesis Contract Review Committee, NCI.

Member, Experimental Therapeutics Contract Review Committee, NCI.

Member, Breast Cancer Virus Segment Working Group, Special Virus Cancer Program, NCI

Participant, Departmental Pesticide Coordinating Committee, DHEW.

Invited Speaker, Advanced Course on Pesticides and Public Health, Pesticides Office, Environmental Protection Agency.

Publications

Bates, R. R., Eaton, S. D. A., Morgan, D. L., Yuspa, S. H.: Replication of DNA After Binding of the Carcinogen 7,12-Dimethylbenz[a]anthracene. J. Nat. Cancer Inst. 45: 1223-1228, 1970.

Yuspa, S. H., Bates, R. R.: The Binding of Benz[a]anthracene to Replicating and Nonreplicating DNA in Cell Culture. Proc. Soc. Exp. Biol. Med. 135: 732-734, 1970.

Yuspa, S. H., Morgan, D. L., and Bates, R. R.: Growth of Fetal Mouse Skin in Cell Culture and Transplantation to F_1 Mice. J. Invest. Derm. 55: 379-389, 1970.

Nebert, D. W., Bausserman, L. L., Bates, R. R.: Effect of 17-β-Estradiol and Testoterone on Aryl Hydrocarbon Hydroxylase Activity in Mouse Tissues *In Vivo* and in Cell Culture. Int. J. Cancer 6: 470-480, 1970.

Serial No. NCI-4687
1. Experimental Pathology
   Branch, OASDC, Etiology
2. Bioassay Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Experimental Models of Human Childhood Neoplasms

Previous Serial Number: NCI-4656; NCI-4681; NCI-4682; NCI-4650

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:	
Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To develop experimental animal models for the various morphological forms of human childhood cancer; to explore the differences in host response to tumors of the same organ systems induced in various animal species at various times during prenatal and postnatal life; to explore the factors responsible for the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens; and to explore the role of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis during perinatal life.

Methods Employed:

Animals of various species and strains are exposed to carcinogens at various times during pre and postnatal life, and the carcinogenic response in various organ systems is evaluated histologically. Tissue culture techniques are used where necessary to explore the role of interferon and other host defense mechanisms in resistance to carcinogenesis. Organic and biological chemical techniques are employed to determine the effects of modifications in chemical carcinogen structure and metabolic factors involved in the response of host tissues to carcinogens as a function of age.

Major Findings:

Previous studies in this laboratory have shown that animals exposed *in utero* to the carcinogen ethylnitrosourea (ENU) are more susceptible than their parents to the carcinogenic action of this compound on pulmonary tissue, and that this effect is most noticeable in animals which are genetically resistant to pulmonary carcinogenesis. The enhanced carcinogenic effect is manifested by a larger number of tumors appearing after a shorter period of time in response to a given dose of carcinogen, and by a more rapid growth rate of the induced tumors in animals exposed *in utero* in comparison with tumors of the same tissue induced in adult animals of the same strain. Numerous previous studies in the literature have suggested that pulmonary tumors in mice are of relatively low antigenicity. These tumors, however, have invariably been obtained from animals of high sensitivity to pulmonary carcinogenesis. We have succeeded within the last two years in establishing two transplantable lines of pulmonary tumors in mice of strain C3HfB. This strain of mouse is relatively resistant to pulmonary carcinogenesis, both spontaneous and induced, and adults of this strain when exposed to ethylnitrosourea or other carcinogens tend to develop a relatively small number of tumors which grow slowly and generally fail to reach a large size (i.e., a size suitable for transplantation) during the life of the host. However, the offspring of animals treated during gestation develop a larger number of tumors which grow very much more rapidly, and the transplanted tumors referred to above were derived from such transplacentally induced tumors. These lines have been shown to be extremely antigenic in the strain of origin, so strongly antigenic that one line has never been carried successfully in that strain! The tumors are readily transplantable in an F_1 hybrid between the strain of origin and the pulmonary tumor sensitive strain, A. The primary response of the parental strain to transplantation of these tumors may be prevented, and the tumors allowed to become established and to grow progressively by specific immunosuppression of C3HfB mice with antilymphocyte serum, and a secondary response can be demonstrated in the F_1 hybrids by the classical techniques of tumor implantation, subsequent excision, and challenge. These studies suggest that resistance to pulmonary tumorigenesis in mice may have an immunological basis, perhaps related to the ability of resistant strains to recognize as foreign antigens which appear on the surfaces of incipient pulmonary tumors, an ability which may be lacking in mice belonging to genetically sensitive strains.

A second series of experiments reported previously from this laboratory have demonstrated that lymphocytic leukemia, induced in Swiss mice by a single exposure during adult life to ENU, can in some cases undergo regression, with the concomitant development of aplastic anemia. Further studies carried out during the past year on this phenomenon have demonstrated that the lymphoproliferative condition which undergoes regression is unequivocally neoplastic, because lymph nodes excised from animals during the florid phase of the disease can be explanted into newborn mice of the same strain, in which they produce a progressive, repeatably transplantable, widespread and fatal lymphocytic leukemia. It has also been documented that this same phenomenon occurs in ENU-treated leukemic mice of strain NZW. Transplantable tumors have been established from animals of this strain, and from an inbred strain derived from the Swiss mouse, designated NGP. These tumors were derived from animals which showed histological evidence of an incomplete form of this regression phenomenon. Experiments are currently under way in order to ascertain the nature of this host response to chemically induced leukemia. Further studies are designed to determine whether or not this defense mechanism is available to mice exposed to the same carcinogen in the same dosage in utero. Studies are under way at the present time in which the effect of dividing the dose of ENU, or of using other carcinogens in its place, will modify the incidence of the regression phenomenon among those animals which develop leukemia as a result of treatment.

A third phenomenon encountered during the past year concerns the development of a variety of highly malignant sarcomas which develop at the site of injection in mice which have received DEAE-dextran subcutaneously. This soluble polymer was originally injected into a series of animals in studies designed for exploration of the antitumor effect of complexes of this material with the synthetic RNA interferon inducer, poly I:poly C. Subsequent studies have shown that sarcomas develop only in mice given the positively charged polymer; uncharged polymer or negatively charged polymer is ineffective, and animals which have been pretreated with the carcinogen ethylnitrosourea by another route prior to injection of the polymers develop a higher proportion of local sarcomas at the site of polymer injection. DEAE-dextran has been used by virologists for some years to enhance the infectivity of a variety of viruses for cell culture, among them the murine sarcoma viruses. It seems not improbable that the mechanism of carcinogenesis by this material may involve enhancement of a latent murine sarcoma virus infection, and studies are being initiated in collaboration with virologists to search for the presence of such an agent in tissue cultures and transplant tumors derived from these neoplasms.

Significance to Biomedical Research and the Program of the Institute:

Research for animal models of human childhood neoplasms should provide an insight into the types of causative agent and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fatal neoplasms before attaining reproductive age. Yet the incidence of tumors in childhood is high, and an increased understanding of the factors responsible for circumventing the innate resistance to oncogenesis in members of a genetically resistant population during prenatal and early postnatal life may be of importance. Studies of transplacental carcinogenesis in mice with one carcinogen have already provided some clues to ways in which genetic resistance to the development of neoplastic disease may be overriden.

Proposed Course:

The proposed course of this project is in two parts. The first envisions an expansion of research for models of human tumors characteristically appearing in childhood. There is at present no model of the embryonal tumors often seen in children, including Wilm's tumor and neuroblastoma. The advantage to the Institute of securing models of such neoplasms consists in the concomitant aquisition of knowledge concerning at least one method by which such tumors can be induced, and therefore a possible insight into the mechanisms of genesis of these tumors in human beings. A special area of investigation of interest is carcinogenesis in utero, by transplacental exposure to carcinogens circulating in the mother's blood stream. The high incidence of tumors in childhood, including occasional congenital neoplasms, suggests that transplacental exposure to chemicals may be an important route for human exposure to carcinogens. The experimental models which are currently available, however, have been incompletely explored. The only really effective transplacental carcinogen known at present is ethylnitrosourea. Furthermore, studies of its effects in mice and rats, although they have produced models of tumors of the central nervous system and of the hematopoietic tissues, have not produced any embryonal tumors and have not succeeded in generating any tumors in mice exposed during early fetal or embryonic life. It is not known whether this represents a failure of the compound to reach susceptible cells, a resistance to such cells to chemical neoplastic transformation, or whether this leads inevitably to tumors of such bizarre characteristics that they are eliminated by host defense mechanisms before they attain significant size. Further exploration of these aspects of carcinogenesis will require a contract-operated facility for the housing of animals of several species in significant numbers, and additional personnel with experience in embryology and pathology who can assume a significant amount of responsibility for these programs.

A second factor related to the first is the matter of increased susceptibility to carcinogenesis during perinatal life as a result of the abrogation of defense mechanisms available to the adult. Approaches to these problems have already been developed, as indicated in the "Findings" section above. These should be pursued, until the phenomena are fully understood. The participation of expert collaborators in fields of virology, immunology, and other areas should be sought as necessary to achieve this goal.

Another factor suggested by present studies on transplacental carcinogenesis is the question of whether tumors which are characteristically those of adulthood or even of old age may arise as a result of exposure to carcinogens during fetal or neonatal life. It is certainly true that in the mouse a higher percentage of tumors which are morphologically of the adult type are induced in animals exposed transplacentally or neonatally to ethylnitrosourea. The doses of carcinogen used in previous studies have generally been large enough to give rise to a high multiplicity of tumors. It is reasonable to expect that if this dose is lowered, and if it is administered at the stage in fetal life when the susceptibility to, e.g., pulmonary carcinogenesis, is greatest, a significant proportion of animals will develop a solitary, large, rapidly growing and highly malignant tumor which would bear a much closer resemblance to the human situation than any studies yet carried out. Exploration of this aspect of transplacental carcinogenesis is also dependent for its success on the availability of contract-supported animal facilities and personnel.

Serial No. NCI-4617
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Carcinogen Screening Operations

Previous Serial Number: Same

Principal Investigators: J. H. Weisburger, Ph.D. and E. K. Weisburger, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	0.7
Other:	0.6

Project Description

Objectives:

Management responsibilities are assumed on the design, performance and evaluation of studies in contract laboratories on the chronic effect and of the carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvement in sensitivity, speed and economy.

Methods Employed:

Attempts are made to secure information on chemicals representing the greatest hazard to the greatest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols (see Weisburger and Weisburger, Methods in Cancer Research, Vol. 1, p. 307) as well as newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized.

Major Findings:

Several of the test series described last year are now reaching the stage of data generation. It was found that a number of drugs, industrial pesticides and other environmental agents, including some natural products, are carcinogenic.

1. Drugs. Drugs now used in the clinic in cancer chemotherapy were active in mice and in rats: Melphalan, chlorambucil, uracil mustard, Natulan or procarbazine, dimethyltriazinoimidazole carboxamide; BCNU and CCNU. These findings are of interest inasmuch as they predict a possible hazard after extensive use in man, if long-term survival of the patient is expected. Primary cancers were induced in man treated with chlornaphthazine some years ago. Recent reports reveal that Melphalan likewise successfully placed patients in remission for one type of cancer, only to cause another type years later. The results being generated at present should assist clinicians to select the most efficient drug from the point of view of the therapeutic effect, and at the same time, constituting the lowest possible long-term risk.

2. Industrial chemicals. Final data with propylenimine and propane sultone, reported in a preliminary way last year, are now available. These two industrial compounds are highly carcinogenic with the interesting and unusual feature that they give rise to gliomas (brain tumors) in high yield relatively quickly. The common commercial compound *o*-toluidine, led to subcutaneous hemangioma in mice (despite oral intake), and probably (to be confirmed) to breast tumors in rats. Likewise, 4-chloro-*o*-toluidine appears to be carcinogenic in mice and in rats. 2,5-Dimethoxy-4'-aminostilbene, advertised in the chemical press as a useful intermediate for large-scale syntheses, gave a high yield of ear duct tumors in rats.

3. Natural products. In the search for natural occurring hazardous materials, a number of fungi were found to lead to pulmonary tumors and leukemia in mice. These include strains of Candida parapsilosis and Candida albicans. Of importance is the finding that aflatoxin, a widespread mycotoxin found in foodstuffs in certain areas of the world, is still carcinogenic even when fed at levels as low as 15 ppb to rats. Further efforts to define the lowest active dose are underway. Cyclopropenoid fatty acids, formerly present in cotton seed oils, potentiate the effect of aflatoxin and also of diethylnitrosamine. Because of this discovery, the industry has now taken measures to eliminate cyclopropenoid acids from cotton seed oil.

4. New analytical method for important aflatoxin metabolite, as indicator of aflatoxin intake. A new metabolite of aflatoxin, the O-demethyl derivative, was identified in several species. This particular metabolite accounts for a substantial fraction of a dose of aflatoxin, in contrast to the earlier metabolites described. The application of a new improved method to assess the presence and quantity of the O-demethyl derivative may permit a new approach to assessing the dietary load of aflatoxin in human populations by analyzing urines for this material. Even taken into account the long latent period between consumption of carcinogens and the eventual development of

clinically identifiable cancer, there is hope that application of the method in countries, where liver and other cancers is suspected to be due to aflatoxin and related materials, will aid to determine definitely whether there is a connection between disease and mycotoxin consumption.

5. Are nitrosamines involved in human cancer? Recently nitrosamines of a variety of structures were found to be carcinogenic in many species. The question thus arises whether such materials are present in the human environment and can account for the induction of some types of human cancer. New specific and sensitive methods for certain types of nitrosamines have been developed. As little as one ppb can be reliably measured as pure chemical, and five to ten ppb in a complex mixture such as a foodstuff. Furthermore, certain nitrosamines can be formed in vivo by the proper combination of nitrite and select secondary amines. Staff has participated in frequent discussions with other government agencies, such as FDA, and other interested parties to keep informed and to contribute to this rapidly moving field and evaluate the potential hazard to man of such possible combinations.

6. Chronic effect of artificial sweeteners. Along these same lines, together with the Associate Scientific Director for Carcinogenesis, the staff of the Section organized a workshop assembling individuals from government, academic and industrial laboratories from the United States and Canada concerned with studies on the safety of artificial sweeteners. Additional contacts in this field are maintained by correspondence with groups in Europe and the Far East. Thus, coordination on an international scale of efforts in this area is being achieved. Tests on saccharin and, for comparative purposes, on cyclamate are underway, and have reached approximately the half-way point in two laboratories under contract to NCI.

Significance to Biomedical Research and the Program of the Institute:

Many factors in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project:

Continuing efforts are required 1. to assess relative priorities of materials to be tested, 2. to undertake studies on variations in the protocols to simplify or to develop test techniques which are more sensitive, more specific and more economic, 3. and to apply such tests to identify carcinogenic hazards in the environment.

Honors and Awards

J. H. Weisburger:

PHS Meritorious Service Medal

Associate Editor, CANCER RESEARCH, 1969-1972

Member, Nominating Committee, American Association for Cancer Research

Member, Editorial Committee, CANCER RESEARCH, American Association for Cancer Research

Member, Editorial Board, FOOD AND COSMETICS TOXICOLOGY

Member, Editorial Board, CHEMICO-BIOLOGICAL INTERACTIONS

Member, Editorial Board, XENOBIOTICS

Member, Advisory Committee and Chairman of Session, 24th Annual Symposium on "Environment and Cancer", M.D. Anderson Hospital and Tumor Institute, Houston, Texas, 1971.

Member, FDA-USDA-PHS Interdepartmental Technical Panel on Carcinogens (through Spring 1971)

Invited Lecture: J. H. Weisburger: Frontiers of Cancer Research, Medical College of Georgia, Augusta, Georgia, May 20, 1970.

Invited Lecture: J. H. Weisburger: New Views on Mechanisms of Carcinogenesis, Particularly with Respect to Aromatic Amines. Canadian Food and Drug Directorate, Ottawa, Canada, June 24, 1970.

Invited Lecture: J. H. Weisburger: Mechanisms of Aromatic Amine Carcinogenesis. New York University Medical Center, Institute of Environmental Medicine, Sterling Forest, New York, August 5, 1970.

Invited Lecture: J. H. Weisburger: On the Mechanisms of Chemical Carcinogenesis, Particularly by Aromatic Amines. First Congress of the European Association for Cancer Research, Brussels, Belgium, September 14-17, 1970.

Invited Lecture: J. H. Weisburger: Die Bedeutung chemischer Carcinogene für die Krebsforschung und die Krebsverhütung. German Cancer Symposium, Heidelberg, Germany, September 23-25, 1970.

Invited Lecture: J. H. Weisburger: Mechanisms of Carcinogenesis and the Prevention of Cancer in Man. Chester Beatty Research Institute, London, September 1970.

Invited Lecture: J. H. Weisburger: Principles and Practice of Industrial Toxicology. Wayne State University, Detroit, Michigan, October 20, 1970.

Invited Lecture: J. H. Weisburger: Le cancer experimental et la prevention du cancer dans l'homme. Institut du Cancer, University of Louvain, Belgium, October 1970.

Invited Lecture: J. H. Weisburger: Neues zur Krebsauslösung durch Arylamine und Nitrosamine. Pharmakologisch-Toxikologisches Kolloquim, University of Tübingen, Germany, October 1970.

Invited Lecture: J. H. Weisburger: Chemical Carcinogens and Their Mode of Action in Colonic Neoplasia. National Conference on Cancer of the Colon and Rectum, San Diego, California, January 7-9, 1971.

Invited Lecture: J. H. Weisburger: Relationship between Experimental Models and the Reality of Carcinogenesis in Man. Symposium on Fundamental Cancer Research, M.D. Anderson Hospital and Tumor Institute, The University of Texas, Houston, March 3-5, 1971.

Publications

Ulland, B., Finkelstein, M., Weisburger, E. K., Rice, J. M., and Weisburger, J. H.: Carcinogenicity of industrial chemicals propylene imine and propane sultone. *Nature* (*in press*).

Weisburger, J. H.: Cyclamates. *Chem. Brit*. (*in press*).

Weisburger, J. H.: Possible carcinogenic hazards of nickel-chelate drugs. *Lancet* i: 401, 1971.

Weisburger, J. H., Klein, M., Weisburger, E. K., Glass, R. M., Woodard, G., and Cronin, M. T. I.: Comparison of the effect of the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide in infant and weanling rats. *J. Nat. Cancer Inst*. 45: 29-35, 1970.

Weisburger, J. H. and Rall, D. P.: Do animal models predict carcinogenic hazards for man? In *Environment and Cancer*, 24th Annual Symposium on Fundamental Cancer Research, M.D. Anderson Hospital and Tumor Institute (*in press*).

Weisburger, J. H. and Weisburger, E. K.: Chemical carcinogens, cancer research, and the prevention of cancer. Proceedings of the Third Heidelberg Symposium, September 1970 (*in press*).

Serial No. NCI-4618
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mode of Action of Chemical Carcinogens -- Endogenous and Exogenous Factors in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: R. S. Yamamoto, Sc.D., G. M. Williams, M.D., E. K. Weisburger, Ph.D., and R. Kroes, D.V.M., Ph.D.

Cooperating Units: B. Zbar, M.D., Biology Branch, NCI

Man Years:	
Total:	3.5
Professional:	1.4
Other:	2.1

Project Description

Objectives:

Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal in the development of systems which lead to the prevention of the processes of chemical carcinogenesis in a study of the mechanisms related thereto.

Methods Employed:

Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically tailored structures or properties are given together with chemical carcinogens to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings:

1. Toxicity and carcinogenicity of new drugs.

Two drugs with promising properties in the chemotherapy screen of CCNSC were studied for carcinogenicity. They were made available by Dr. C. T. Bahner of Carson-Newman College. 1-(4-N-Nitroso-N-methylaminobenzylidene)indene fed at a level of 800 ppm in the diet to male and female rats for 48 weeks led to tumors in a number of organs, including liver and ear duct. A compound with a similar structure, 4-(4-N-nitroso-N-methylaminostyryl)quinoline, fed at 25 ppm, likewise was highly carcinogenic.

2. Effect of 8-hydroxyquinoline on the carcinogenicity of N-2-fluorenylacetamide and of diethylnitrosamine. Failure of iron to localize in hyperplastic nodules.

In connection with studies on the chronic toxicity of the drug and industrial compound, 8-hydroxyquinoline, it was noted that under our conditions, 8-hydroxyquinoline administration in the diet led to iron deposition (hemosiderosis) in many organs, but particularly in spleen and liver. It was also noted that 8-hydroxyquinoline delayed the tumor formation with the carcinogen N-2-fluorenylacetamide. The interesting observation was made in connection with these studies that those animals bearing hyperplastic nodules and/or hepatocellular carcinomas, failed to exhibit localization of iron in the preneoplastic tissues, whereas the surrounding liver did concentrate iron.

Further study of this process with a different liver carcinogen, diethylnitrosamine, also documented the fact that the hyperplastic nodules with this agent also acted likewise. The exact mechanism requires further investigation but appears to be of interest inasmuch as liver tumors and even hyperplastic nodules do not bind other essential exogenous and endogenous metabolites. The iron deposition mediated by 8-hydroxyquinoline is a useful tool to study this process in detail.

In connection with these studies, the unexpected and interesting observation was made that dietary administration of 8-hydroxyquinoline severely inhibited the liver tumor formation with diethylnitrosamine. As mentioned elsewhere in these reports, the nitrosamine type of compound has attracted attention of late as being a ubiquitous contaminant of the environment. Consideration must be given that such agents may be involved in the genesis of human cancers. Such compounds are active under many different conditions, in virtually all species in which they were tested. The reason for this broad activity spectrum is that the proximate or ultimate carcinogen is generated from the precursor materials by enzymes present in many tissues of many species. There are few known inhibitors with low mammalian toxicity of these particular enzymes. Thus, the discovery that 8-hydroxyquinoline does inhibit the carcinogenicity of one typical nitrosamine deserves further study and extension to other agents of this type.

3. Further evidence that sulfate esters are the crucial ultimate carcinogens derived from carcinogenic aromatic amines.

A key discovery was reported in a preliminary way last year: Sulfate esters of the N-hydroxy derivatives of aromatic amines are the significant ultimate carcinogens for liver cancer formation derived from aromatic amines. This was documented unambiguously by taking advantage of a inhibitory system developed in this laboratory some time ago. An excess of acetanilide fed together with a typical aromatic amine like *N*-hydroxy-*N*-2-fluorenylacetamide inhibited toxicity and the carcinogenicity to the liver. The underlying reason was a depletion of endogenous available sulfate by *para*-hydroxyacetanilide, the main metabolite of acetanilide. We have now extended these observations by showing that increasing the dietary sulfate to an equimolar level or three times the equimolar level to the acetanilide utilized, restored the carcinogenicity of *N*-hydroxy-*N*-2-fluorenylacetamide. The three-fold excess of inorganic sulfate appears to be more effective. Another potential ester of the N-hydroxy derivative, the phosphate ester, does not appear to be involved in carcinogenicity, for substitution of phosphate failed to restore the blocked carcinogenic process when acetanilide or *para*-hydroxyacetanilide were administered.

4. Effect of antioxidants on the carcinogenicity of N-2-fluorenylacetamide or N-hydroxy-N-2-fluorenylacetamide.

Theoretical reasons suggested that antioxidants might interfere with the carcinogenic process, either by preventing certain of the required activation reactions of the chemical, or by acting as a trapping agent for the active carcinogenic intermediate.

Vitamin C, a powerful antioxidant and chelating agent administered in the diet at a level of 1% to rats, however, failed to affect the carcinogenic process induced by 150 ppm *N*-2-fluorenylacetamide or the equimolar amount 160 ppm *N*-hydroxy-*N*-2-fluorenylacetamide.

Success was achieved, nonetheless, when the commercial antioxidant BHT (butylated hydroxytoluene), utilized by the food industry, was administered at a 5% level in the diet to rats also fed the carcinogens mentioned above, and inhibited the development of liver tumors in rats. The mechanism requires further investigation. Data show that it does not appear to hinge on a depletion of sulfate ions, which might be possible on the basis of the similar action of *para*-hydroxyacetanilide. However, additional preliminary results in a collaborative contract laboratory, while confirming the effect of BHT also shows that another antioxidant, DPPD (diphenyl-*p*-phenylenediamine), fails to affect the carcinogenic process under comparable conditions.

5. Investigation of new species for carcinogen screening.

The standard species for the investigation of the environmental hazards, especially carcinogenicity are chiefly mice, rats and hamsters. New species would be desirable if they would respond more rapidly to a given carcinogenic challenge and also if they would exhibit a low spontaneous tumor rate. Scientists at the Armed Forces Institute of Pathology reported that the African white-tailed rat, Mystromys albicaudatus, was a small, relatively, easily handled animal with good reproductive capacity and, above all, a low spontaneous tumor incidence. The response of Mystromys to a standard treatment with 40 ppm diethylnitrosamine in drinking water was assessed in comparison to rats. The latter develop extensive liver cancer with the regimen administered for 10 weeks and autopsied 10 weeks later, or after a total of 20 weeks. At that point, even though the treatment was maintained throughout the experiment, mystromys exhibit little damage. It is only after approximately one year that they present liver and possibly stomach tumors (histologic study now underway). We also noted that old control Mystromys show fairly extensive palillomatous structures in the forestomach. On the basis of these tests, it would appear that Mystromys do not meet the requirements for a good carcinogen screening system.

6. Is benzene leukomogenic in animal systems?

Case reports suggest that under some conditions benzene, the widely used solvent and an ingredient of petroleum products, has led to leukemia in man. There are no good documented animal studies to support this activity. With other agents, C57BL mice, particularly males, appear to be a useful system to detect leukomogenic activity. Thus, benzene is being injected subcutaneously into C57BL mice. Some mice that have come to autopsy at about one year have exhibited anemia and an altered blood picture. However, thus far no leukemia has been observed. These tests are continuing.

7. Inhibition of mammary tumor formation in C3H mice.

In the course of assessing the chronic effect of hydroxylamine, an industrial product which is mutagenic and teratogenic under some conditions, we found that this material was not carcinogenic (Proc. Soc. Exp. Biol. Med. 124: 1217, 1967). On the contrary, we found hydroxylamine inhibited the mammary tumor formation resulting from the action of the endogenous mammary tumor virus. Examining the mechanism of this process, it was now noted that inhibition is obtained only when the hydroxylamine treatment is begun with animals at weaning. Initiation of the hydroxylamine treatment at 6, 9 and 12 months fails to inhibit mammary tumor formation. On the other hand, hydroxylamine does not prevent the spontaneous leukemia development in AKR mice.

8. The immunosuppressive effect of griseofulvin in mice.

Griseofulvin is a most improtant drug, extensively used in dermatology. In clinical application griseofulvin is administered to patients orally in large doses for long periods of time. Therefore, some reports in the literature that it might be carcinogenic were highly disturbing. Chronic administration of griseofulvin to mice of strains exhibiting a low spontaneous tumor rate in the liver gave rise to a high incidence of hepatoma after less than one year on test. Rats tested so far were negative. In another series of assays, oral but not cutaneous griseofulvin potentiated the effect of a carcinogenic hydrocarbon applied to the skin of mice. These experiments suggested to us that griseofulvin was perhaps not a carcinogen as such, but acted under conditions where potentiation of an existing process took place by virtue of a immunosuppressive effect. This point was investigated in experiments performed with Dr. B. Zbar of the Biology Branch.

Griseofulvin was fed to mice at 1% dietary level for 4 or 8 weeks. At this point the effect of griseofulvin on the immune response of mice to infection with *Listeria monocytogenes* was evaluated. The nature of the immune response of mice to these bacteria has been well characterized. The kind of immune response that occurs is of the cell-mediated, delayed hypersensitivity type, thought to be of paramount importance in rejection of tumor grafts. Adult mice fed griseofulvin were found to have an impaired response to infection with *L. monocytogenes*. This impaired response was measured by determining the number of bacteria in the spleens of experimental and control mice at various times after infection. Newborn mice receiving griseofulvin by intraperitoneal injection were not found to have an impaired response to *L. monocytogenes*, when tested when the mice were 30 and 60 days old.

Significance to Biomedical Research and the Program of the Institute:

The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project:

A number of the tests for chronic toxicity and carcinogenicity presently underway or planned to go on stream shortly will require considerable effort for several years. Additional emphasis will be placed to find systems of fundamental value which will give insight into methods for inhibiting the carcinogenic process and to understand the various steps leading to such inhibitions. Thus studies will be variations in endogenous factors as well as select exogenous materials.

Publications

Frankel, H. H., Yamamoto, R. S., Weisburger, E. K., and Weisburger, J. H.: Chronic toxicity of azathioprine and the effect of this immunosuppressant on liver tumor induction by the carcinogen N-hydroxy-N-2-fluorenylacetamide. Toxicol. Appl. Pharmacol. 17: 462-480, 1970.

Weisburger, J. H.: Host-dependent factors in environmental carcinogenesis. In Environment and Cancer, 24th Annual Symposium on Fundamental Cancer Research, M.D. Anderson Hospital and Tumor Institute (in press).

Yamamoto, R. S., Frankel, H. H., and Weisburger, J. H.: Effects of isomers of acetotoluidine and aminobenzoic acid on the toxicity and carcinogenicity of N-2-fluorenylacetamide. Toxicol. Appl. Pharmacol. 17: 98-106, 1970.

Yamamoto, R. S., Weisburger, J. H., and Weisburger, E. K.: Controlling factors in urethan carcinogenesis in mice: Effect of enzyme inducers and metabolic inhibitors. Cancer Res. (in press).

Serial No. NCI-4619
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mode of Action of Chemical Carcinogens -- Studies on the Metabolism of Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: P. H. Grantham, M.S., T. Matsushima, Ph.D., L. Bahna, M.D., Ph.D., E. K. Weisburger, Ph.D., and N. B. Giao, D.V.M., Ph.D.

Cooperating Units: Laboratory of Chemistry, NHLI (Dr. Fales)

Man Years:

Total:	4.7
Professional:	2.8
Other:	1.9

Project Description

Objectives:

The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors and accelerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed:

Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide and related compounds in various animal species. This includes isolation and purification of enzyme systems concerned with certain metabolic steps.

Procedures are developed and applied for the separation, purification and analysis of macromolecular constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals.

The metabolism of chemical carcinogens and interaction with host targets is examined *in vivo* and *in vitro* in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings:

1. Intestinal flora as a modifier of the metabolism of some chemical carcinogens.

We reported previously that the microbiological flora in the lower gut of rats participated in the overall metabolism of some chemical carcinogens.

Further detailed questions were asked of this system. It was shown that β-glucuronidase from certain of the bacteria in cecum hydrolyzed carcinogen metabolites entering the gut as conjugates *via* the bile. Water-soluble conjugates such as glucosiduronic acids, glycosides, ureido-derivatives, and the like, are poorly absorbed from the intestinal tract. However, after hydrolytic splitting by bacteria in the lower gut, the more lipid-soluble materials released are more readily absorbed. Thus, the enterohepatic cycle involves conjugation in organs such as the liver, excretion in bile, and metabolic splitting of conjugates by bacteria in the gut followed by reabsorption of the products, rather than circulation of unchanged materials. Thus, materials cleared poorly *via* the renal pathway may remain in the host for a extended time and, therefore, explain the long-acting effect of certain drugs. In addition to β-glucuronidase, other hydrolytic enzymes such as sulfatases are also present in the bacterial flora.

In addition to hydrolytic enzymes, we have demonstrated the presence of azo dye reductases and another interesting reducing system which converts the active metabolite derived from *N*-2-fluorenylacetamide, the *N*-hydroxy derivative back to the parent compound. This reaction is a form of detoxification. Single strains of bacteria, isolated from the cecal contents or feces of rats, which possessed dehydroxylating activity were E. coli, A. aerogenes, B. proteus, P. aeruginosa and B. faecale alcaligenes. Furthermore, a large number of strains of bacteria were isolated and tested for capability to deacetylate *N*-2-fluorenylacetamide. At least two strains had this activity, B. faecale alcaligenes and A. aerogenes. Interestingly, the deacetylating capability was found only at 37°C, not at 30°C. Also, the enzyme activity was present chiefly under aerobic conditions and was suppressed by media containing dextrose. Two species which can generate hydrogen sulfide, B. proteus vulgaris and Citrobacter, also N-dehydroxylated in the presence of dextrose, suggesting that in this case the reduction was mediated by hydrogen sulfide, in turn produced from sulfur containing amino acids under anaerobic conditions.

Another question asked dealt with the nature of the enzyme reducing the hydroxylamino derivative of the carcinogen, in relation to enzymes in bacteria which reduce nitrate to nitrite to hydroxylamine to ammonia, the well-known nitrogen cycle, which in turn may be involved in the production of carcinogenic nitrosamines from nitrate and certain secondary amines. We found that the enzyme capability of bacterial strains to participate in the inorganic nitrogen cycle was probably not identical to those which reproduced N-hydroxy-N-2-fluorenylacetamide to the parent compound. However, further investigations with these two systems may be useful in connection with our parallel studies on the etiology of colon cancer (NCI-4688).

Attempts were made to isolate the various enzyme systems from bacteria so as to secure better evidence on their identity or non-identity among themselves and also with similar components of mammalian systems. However, disintegration of bacterial flora by sonication and by other means led to complete loss of enzyme activity. Thus, other ways must be found to release the enzymes from the bacteria.

2. Water-soluble metabolites of N-hydroxy-N-2-fluorenylacetamide.

Efforts have continued to identify the water-soluble metabolites found in urine after doses of N-2-fluorenylacetamide or the N-hydroxy derivative. This difficult problem has not yet been completely resolved. It would appear, however, that at least one of the free metabolites might contain sulfur in some form. Resolution of the nature of these metabolites would contribute to our knowledge of the drug metabolism generally, because in almost all drug metabolic studies, such materials are found, and have thus far not been satisfactorally identified. It seems reasonable to assume that such materials participate in the biological action of drugs and carcinogens.

3. Biochemical studies on sulfuric acid esters, crucial carcinogenic intermediates.

Under NCI-4618 we discussed the biologic experiments proving that sulfate esters are the ultimate carcinogens for liver cancer causation derived from N-hydroxy-N-2-fluorenylacetamide. In the course of these tests, animals were examined for the excretion of free, conjugated and total sulfate so as to document in this fashion the fact that sulfate esters are actually the key intermediates. That this is so was proved, since the groups of animals receiving acetanilide and the carcinogen exhibited very low levels of free urinary sulfate and most was in conjugated form with para-hydroxyacetanilide. On the other hand, in the group not receiving acetanilide, or those groups receiving acetanilide and supplements of sulfate, some free sulfate was excreted, proportional to the amount of dietary sulfate. Thus, the level of tissue sulfate was sufficient to permit operation of the sulfotransferase-PAPS sulfating system.

4. Are there differences in the metabolism of a drug if given as a single acute dose, or is fed for one day or chronically?

It has been known for some time that the metabolic fate of carcinogenic aromatic amine derivatives such as N-fluorenylacetamide depends in part on the mode of administration. The quantitative distribution of metabolites excreted in urine and present in tissues is quite different when the material is administered as a single ip dose compared to when it is fed in the diet for a number of weeks. We have demonstrated that non-carcinogenic acetanilide inhibits the carcinogenicity of N-2-fluorenylacetamide under chronic conditions. Previous data showed that the rationale for the inhibition could rest on two phenomena, 1. lowering of available sulfate and 2. competitive binding at the level of cellular and molecular receptors. Hence, it was judged important to examine the metabolism of acetanilide under chronic conditions.

Thus, metabolites of acetanilide present in the excreta circulating in the blood and found in tissues, particularly those metabolites bound to molecular receptors were studied after a single ip dose and upon continuous feeding in the diet for one day, one week, and four weeks. In the single dose compared to the more chronic treatment, there were more deacetylated metabolites. During the chronic treatment higher levels of glucosiduronic acids but lower levels of sulfate ester metabolites were found. Plasma and red cell binding was much higher after chronic administration than after the injection procedure. At the intracellular level, the highest of binding was seen to ribosomal RNA and somewhat lower levels to cellular proteins. However, little difference was seen as a function of frequency of administration. With labelling of DNA, however, higher levels were seen after four weeks intake, compared to the single dose treatment when only very low levels were found. In this connection, the loss of label to these various cellular and molecular fractions was studied upon return to control diet, subsequent to a course of four weeks of labelled acetanilide. The activity bound to the plasma fractions as well as the soluble proteins, soluble and ribosomal RNA in liver decayed with a half time of approximately four and one-half to five days. Radioactivity bound to DNA disappeared more slowly.

A number of important conclusions can be drawn from an evaluation of the combined results of these extensive experiments. We demonstrate with a non-carcinogenic drug that the alteration in metabolism, previously demonstrated with a carcinogen, when material is administered chronically compared to a single dose may well be a general phenomenon. Most studies in pharmacology examine the metabolism of drugs after a single dose, which appears suitable when a drug is thus administered. However, when drugs are used under more chronic conditions erroneous conclusions could be drawn if the metabolism was examined only after an acute dose. More specifically related to the present question, from the fact that acetanilide itself does bind to DNA, suggests that this may also play a role in the inhibitory action this material exerts on the carcinogenicity of N-2-fluorenylacetamide, even though quantitatively the binding of acetanilide is much lower than that of the carcinogen.

5. Properties of the microsomal enzyme systems yielding active carcinogenic intermediates.

The essence of our preliminary findings in this area were discussed last year. By means of classic inhibition experiments we demonstrated that the key activation reaction of a carcinogenic aromatic amine such as N-2-fluorenylacetamide, namely N-hydroxylation, occurred under conditions somewhat different from the detoxification reactions, the various ring- or carbon-hydroxylation reactions. Additional tests confirm the concepts derived from the preliminary studies. Utilizing aminotriazole as inhibitor of biochemical hydroxylation in hamster liver microsomes, it was found that the base level of N-hydroxy derivative was increased when the inhibitor was injected, whereas ring-hydroxylation was decreased. On the other hand, in animals pretreated with an enzyme inducer such as methylcholanthrene, or phenobarbital, all microsomal hydroxylation reactions were depressed by simultaneous exposure to aminotriazole.

In view of the detailed differences noted in hydroxylation capability as a function of substrate, environmental conditions and the presence or absence of inducers and inhibitors, it now seems urgent to attack the problem of isolating and identifying the relevant hydroxylating systems involved. If the study of the specific isolated systems confirms their distinct nature, the significant goal of selective inhibition of enzymes performing activation reactions, in contrast to those performing detoxification reactions may prove feasible.

In addition to these *in vitro* studies, a series of *in vivo* experiments utilizing phenobarbital as an enzyme inducer and N-2-fluorenylacetamide as substrate were performed. Despite an increased excretion of the active metabolite N-hydroxy-N-2-fluorenylacetamide, it was found that label from the carcinogen was bound much less to DNA and other molecular receptors than in untreated control animals. Two explanations were possible. Phenobarbital might affect the sulfotransfurase activity required for further activating the carcinogen, or alternatively phenobarbital administered in large amounts could act as a trap for sulfate ions required for the activation reaction. Experimental tests of these two possibilities did not confirm either one. Additional explanations need to be examined inasmuch as the lower DNA binding appears significantly related to a lower carcinogenic potency in rats simultaneously given the carcinogen and phenobarbital.

6. The metabolism of 6-aminochrysene.

6-Aminochrysene is a drug developed by Professor Buu-Hoi and associates in Paris for two applications and use in man. 1. It reduces splenomegaly secondary to malaria; 2. it appears active against human breast cancer. Chronic studies by Buu-Hoi and associates in a small number of animals suggested that 6-aminochrysene was not carcinogenic. However, Rowe and associates in London found that hepatomas were produced when the drug was injected into newborn mice. Inasmuch as it seems fairly certain that carcinogenic aromatic amines require N-hydroxylation followed by conjugation of the resulting metabolite, metabolism studies were undertaken to answer the question whether this does occur with 6-aminochrysene, in collaboration with Dr. Buu-Hoi of Paris and Dr. Roncucci of Brussels who supplied the isotopically labeled drug. Results obtained thus far indicate that of a given dose of the compound, 20% is excreted in urine and about 60-80% in the stools. The excretion occurs slowly, suggesting there is extensive recirculation, possibly via the enterohepatic cycle. Most of the fecal metabolites are free compounds, whereas in urine free compounds and a number of conjugates are seen. The free compounds in turn have resolved thus far into four distinct metabolites. Additional work is required to perfect the methodology of fractionation and analyses of the various metabolites so that these techniques can be routinely applied to study the metabolites in a variety of species under several different conditions.

Significance to Biomedical Research and the Program of the Institute:

By the utilization of certain chemical carcinogens, the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps going from 1. the primary interaction between an agent and a crucial cellular constituent or constituents, a reaction for which there are repair processes antagonizing the attack, followed by 2. multiplication of abnormal cells which in turn can 3. undergo further transformations, it is easy to visualize a number of points which with future effort could conceivably prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to fully comprehend the sequence of complex reactions leading to cancer and hopefully to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project:

The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Publications

Williams, J. R., Grantham, P. H., Yamamoto, R. S., and Weisburger, J. H.: Effect of dietary riboflavin on azo dye reductase in liver and in bacteria of cecal contents of rats. Biochem. Pharmacol. 19: 2523-2525, 1970.

Weisburger, J. H. and Weisburger, E. K.: N-oxidation enzymes. In Gillette, J. and Brodie, B. (Eds.): Handbook of Experimental Pharmacology (in press).

Serial No. NCI-4620
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mode of Action of Chemical Carcinogens -- Chemical Investigations

Previous Serial Number: Same

Principal Investigator: E. K. Weisburger, Ph.D.

Other Investigators: J. H. Weisburger, Ph.D.

Cooperating Units: Laboratory of Chemistry, NHLI (Dr. Fales)
Viral Carcinogenesis Branch, NCI (Dr. Huebner)

Man Years:

Total:	0.5
Professional:	0.4
Other:	0.1

Project Description

Objectives:

In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens, and in model experiments, on the reaction of chemical carcinogens with select potential targets. The aim is to elucidate fundamental factors involved in chemical carcinogenesis.

Methods Employed:

Properties of chemicals acquired or synthesized for the bioassay of chemical carcinogens are investigated to determine the purity of the materials. In model experiments the reaction of certain of these agents, particularly their primary and ultimate reactive forms with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings:

During the past year compounds needed as reference materials in the study of acetanilide metabolism were prepared. In addition numerous samples of strong and weak carcinogens of various classes and their non-carcinogenic analogs were purified for use by Dr. Huebner in his *in vitro* studies on viral factors in carcinogenesis.

This project continues to furnish standard carcinogens to other investigators and to assist other researchers at NCI who are not equipped to check the purity of their standard carcinogens.

Significance to Biomedical Research and the Program of the Institute:

The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Section.

Proposed Course of Project:

It is hoped that during the coming year, in cooperation with CCNSC, a contract to store and to furnish purified samples of standard carcinogens to various investigators will become operational. This will lessen the need for the service aspects of this project.

Honors and Awards

E. K. Weisburger:

Chemical Society of Washington - Chairman, Community Service Award Committee, 1971

Sigma Delta Epsilon, Omicron Chapter, President, 1970-1971

Editorial Board, JOURNAL OF THE NATIONAL CANCER INSTITUTE; Assistant Editor-in-Chief, February 1971--

Editorial Board, EXCERPTA MEDICA (Cancer)

Member, Chemical Carcinogenesis Contract Review Committee

Elected to Board of Trustees, Lebanon Valley College, 1970-1973

Staff Consultant, CARCINOGENESIS ABSTRACTS

Invited observer, meeting of Toilet Goods Association, Washington, D.C., October 26-28, 1970

Invited speaker, Symposium of Society of Cosmetic Chemists, May 24-25, 1971

Serial No. NCI-4680
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mode of Action of Chemical Carcinogens -- Development and Application of *In Vitro* Systems Involving Epithelial Cells

Previous Serial Number: Same

Principal Investigators: G. M. Williams, M.D. and J. H. Weisburger, Ph.D.

Other Investigators: R. Kroes, D.V.M., Ph.D.

Cooperating Units: None

Man Years:
Total: 2.2
Professional: 1.1
Other: 1.1

Project Description

Objectives:

Neoplasia in man and animals involves mostly epithelial cells. Thus far efforts to grow epithelial cells *in vitro* under conditions required for studies of neoplastic transformation have been mostly unsuccessful. Many efforts along these lines have been at the level of cultures derived from whole embryos or of fibroblasts. Those instances of transformation seen resulted in the production of mesenchymal tumors. Our principal aim was to secure cultures of epithelial cells from liver for studies on their biochemical properties and attempts at transformation. Once established such systems could serve to not only gain insight into the mechanism of action of chemical carcinogens under readily controlled conditions but hopefully also assist in developing quick and reliable bioassay procedures for chemical carcinogens.

Methods Employed:

Classical tissue culture methods were applied but modified in such a manner to select epithelial cells and maintain them under conditions where continuing growth and proliferation was maintained. Procedures were applied to characterize these cells by biochemical and morphological tests. Select carcinogens were added and the results evaluated with reference to specific criteria.

Major Findings:

1. Development and application of epithelial liver cell cultures.

There have been numerous attempts to grow epithelial liver cells *in vitro*, which failed either because the epithelial cells could not be maintained and died out, or because of overgrowth by fibroblasts. Over the last several years we now have proved out our methodology in a reproducible manner, which leads to stable epithelial liver cell lines which continue to proliferate and in which fibroblasts are absent. Several of our lines are now over a year old and continue their growth pattern unaltered. Periodic injection into syngeneic hosts have thus far failed to give rise to any visible tumor, pointing to the absence of spontaneous transformation. We have noted earlier that if precautions are not taken to remove fibroblasts in explants of liver, fibroblasts will not only overgrow the epithelial components but will rather quickly transform spontaneously so that injection into syngeneic hosts will give rise to sarcomas, as discussed last year.

Biochemical evaluation of the epithelial cells demonstrates the presence of an inducible benzopyrene hydroxylase. *N*-2-Fluorenylacetamide can also be hydroxylated, but curiously when measured polarographically, no disappearance of dimethylnitrosamine could be detected. By histochemical techniques the presence of ATPase, alkaline phosphatase, NADP and NAD reductases and non-specific esterases was established. Glucose 6-phosphatase and leucine amino-peptidase was not detectable. Additional efforts to delineate the biochemical potential of this *in vitro* system are now underway.

A number of carcinogens belonging to various chemical classes have led to transformation of these epithelial cells as evidenced by alteration in morphology, in growth rate and in some cases, of the karyotype. Thus far, injection of pools of the transformed cells into newborn syngeneic hosts have failed to give rise to visible tumors. Additional tests are now underway utilizing radiation-treated syngeneic animals. Also, additional chemical carcinogens and related non-carcinogens will be inserted into the system in order to develop a comprehensive view on the potential usefulness of these cells as an *in vitro* bioassay system. Further plans also include the utilization of liver cells from other species, including man, as well as cells from other tissues of contemporary interest, such as the intestinal tract, pancreas, and other digestive and excretory organs.

2. Fetal globulins in liver cancer.

Additional tests were performed to secure a monospecific antiserum so as to be in a position to evaluate the presence or absence of α-fetoglobulins during carcinogenesis with a variety of hepatocarcinogens. A high titer antiserum has been produced, and the next stage involves adsorption processes to secure the desired monospecific antiserum. This program is rather complex and will require additional developmental effort.

Significance to Biomedical Research and the Program of the Institute:

An assay system which can be designed to give results more rapidly, more specifically, and more reliably than the customary long-term tests for carcinogenicity is a highly desirable aim in the field of chemical carcinogenesis with the dual purpose of studying fundamental phenomena and also the detection of harmful agents in our environment. Full exploitation of workable and realistic *in vitro* systems in this respect has not received the attention it deserves. It is our goal to develop such systems with the aim of furthering efforts leading to the prevention of cancer.

Proposed Course of Project:

The important *in vitro* test systems involving epithelial cells will be carefully examined for suitability in carcinogen screening systems. Also the relevant morphological and biochemical properties in normal and carcinogen treated cells will be subject to thorough scrutiny. Also of interest in relation to early diagnosis of neoplastic change will be the *in vitro* and *in vivo* immunological work relative to generation of specific fetal proteins.

Serial No. NCI-4688
1. Experimental Pathology Branch, OASDC, Etiology
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mode of Action of Chemical Carcinogens -- Investigations of Systems Leading to Cancer in Colon and Other Digestive Organs

Previous Serial Number: None

Principal Investigator: J. H. Weisburger, Ph.D.

Other Investigators: R. S. Yamamoto, Sc.D.

Cooperating Units: J. W. Berg, M.D.

Man Years:

Total:	1.8
Professional:	0.6
Other:	1.2

Project Description

Objectives:

In the United States the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in females after breast and uterus. In some other countries like in Western Europe, the relationship is similar. However, in other countries such as Japan and Africa, colon cancer is much lower, thus suggesting that environmental factors are connected with the etiology of this important disease. Cancer of the pancreas is increasing and nothing is known precisely on causative factors. Because of the absence of adequate tools to induce colon and rectal cancer in animals, model studies could not be performed. However, recently several new agents were discovered by which colon and in part rectum cancer can be reproducibly induced in animal systems. There is a possibility that cancer of the pancreas may also result. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon, rectum and perhaps pancreas in animal systems, and thereby gain insight into conditions possibly operating in man.

Methods Employed:

Rats, mice, guinea pigs and also other rodent species were or will be treated appropriately with chemicals which have been shown to lead to colon cancer. They include 1,2-dimethylhydrazine, azoxymethane, methylnitrosourea, and 2',3-dimethyl-4-aminobiphenyl. The animals are placed on diets mimicking those utilized in various parts of the world as regards quantity and quality of protein, fat, carbohydrate, micronutrient and trace element. Other variables examined initially are route of intake of the agent as well as dose levels. Male and female Fischer strain rats were injected twice per week with an aqueous solution of dimethylhydrazine for 16 weeks, then one time per week for another 10 weeks, and were autopsied after a total of 32 and 34 weeks, respectively, for males and females. Groups of rats which were on a commercial chow or a semi-synthetic diet containing 18% protein or the same diet with additional tryptophan or on a high-protein (27%) diet developed colon cancer, as well as, unexpectedly, ear duct cancer. Under the same conditions animals in the high-fat, or high-fat high-protein groups died prematurely, due to toxicity of the carcinogen regimen. It would appear that the carcinogen is less well tolerated in the high-fat groups. Furthermore, the high tryptophan group presented with enlargement and growth of the pancreas and surrounding tissues, an interesting observation deserving additional exploration. Further studies involving lower carcinogen dosage regimens, and also utilizing azoxymethane in rats and in mice are presently underway on all of these dietary conditions.

Inasmuch as induction procedures are being carried forward in inbred strains of rats, the possibility exists to secure transplantable colon cancers for biochemical and immunologic studies. Efforts along these lines are now underway.

Significance to Biomedical Research and the Program of the Institute:

In view of the importance of colon cancer in man, model studies on the mechanisms of colon cancer formation are of paramount importance, especially since epidemiologic and migrant data do indicate that colon cancer almost certainly is due to environmental factors. The systems now studied and related efforts to be developed extensively in the peripheral areas such as the enzymology of the colon and the corresponding tumors, immunological factors including the occurrence of carcino-embryonic antigens, the development of in vitro systems related to colon carcinogenesis and the like should in due time produce an accurate picture of factors controlling colon cancer induction and development so that this important disease in man would eventually yield to realistic preventive efforts.

Proposed Course of Project:

Numerous relevant variables need to be studied in the induction of colon cancer in our model systems. Several other carcinogens must be applied, including some endogenously formed, possibly from a combination of nitrite and an appropriate secondary amine derivative, in order to permit eventual extrapolation to man. Systems must be studied in other species as well. Inasmuch as this is a relatively uncharted road, many different points of attack are possible. On the basis of our past experience with other organ systems such as liver, some selectivity is possible. Also guidance will be obtained from a study of factors favoring the presence of colon cancer in man as a function of the environment, so that model studies will be undertaken which will yield significant information with hopefully a mimimum of waste motion. Inasmuch as the bacterial flora in the gut is almost certainly involved in the development of colon cancer, this element deserves exploration. Rather than attacking the problem indirectly by examining the complex bacterial flora as such, we hope to devise methods to assess the relevant enzyme capability of a given bacterial flora, since the enzymic potential is actually the key element. Further to be considered are germ-free or antibiotic-treated systems, as well as animals deliberately fed diets to provide high or low bulk in the intestinal tract. Similar test series will be directed toward the study of cancer of the pancreas, and indeed other digestive organs, as model systems can be generated.

Publications

Weisburger, J. H.: On colon carcinogens, their metabolism and mode of action. _Cancer_ (_in press_).

III. *CONTRACT NARRATIVES*

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

July 1, 1970 through June 30, 1971

CHEMICAL CARCINOGENESIS CONTRACT REVIEW COMMITTEE

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, ET, NCI, *Committee Chairman*

Mr. Raymond Newell, *Executive Secretary*

Mr. Maurice Fortin, *Contract Specialist*

Dr. Richard Bates, Acting Chief, Experimental Pathology Branch, Carcinogenesis, ET, NCI

Dr. John Berg, Head, Epidemiologic Pathology Unit, Carcinogenesis, ET, NCI

Dr. Thaddeus Domanski, Chief, Awards Review and Technical Administration Branch, EA, NCI

Dr. John Gart, Head, Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Demography, ET, NCI

Dr. Joseph Leiter, Associate Director for Intramural Programs, NLM

Dr. John Mead, Special Assistant for Contract Operations, Experimental Therapeutics, Chemotherapy, NCI

Mr. Samuel Poiley, Head, Mammalian Genetics and Animal Production Section, Chemotherapy, NCI

Dr. Elizabeth Weisburger, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis, ET, NCI

Dr. Harry Wood, Chief, Drug Development Branch, Chemotherapy, NCI

Dr. Roger O'Gara, Laboratory of Pathology, General Laboratories and Clinics, NCI, served on the Committee from December 1967 until his death on January 25, 1971.

ASH STEVENS, INC. (PH43-66-929)

Title: Synthesis of Purine and Pyrimidine Nucleotides

Contractor's Project Directors: Prof. Calvin Stevens
Dr. Arthur Ash

Project Officer (NCI): Dr. Michael Sporn

Objectives: The general aim of this contract is to design and make available a new type of pharmacological agent which will be useful in investigating the manner in which chemical and viral carcinogens affect the metabolism of newly synthesized and messenger RNA in the cell nucleus, as well as a variety of other problems in chemical and viral carcinogenesis. The specific aim of the contract is to synthesize an organic chemical which will irreversibly inactivate the nuclear enzyme which destroys newly synthesized and messenger RNA in the nucleus itself; the chemical agent must be selective and biologically active.

Major Findings: A number of inhibiting groups has been attached to the amino function of phosphorylated 3'-amino-3'deoxythmidine and 5'-amino-5'deoxythymidine. The enzymatic test results indicate that the 3'-isomers are more active exoribonuclease inhibitors than the 5'-isomers. Furthermore, the new compound, 3'-chloroacetyl-amino-3'-deoxythymidine-5'-phosphate, when assayed at 2.5×10^{-3}M, was found to give 60-80% inhibition of exoribonuclease, but no inhibition of RNA polymerase. Thus the goal of selectively inhibiting RNA degradation without inhibiting RNA synthesis has been met in an *in vitro* situation.

Synthetic procedures were developed to synthesize polynucleotides in large quantities. The method utilizes neutral, phenyl triesters which can be purified by large-scale silica gel column chromatography and/or large-scale thin layer chromatography, in contrast to charged diester polynucleotides which must be purified by ppc or anion exchange chromatography. The trityl and acetyl groups are used to protect hydroxyl groups. The trityl group can be removed selectively in the presence of the phenyl ester and acetyl protection; the acetyl group can be removed in the presence of phenyl and the trityl protection; and both the trityl and the acetyl groups can be removed in the presence of the phenyl protection. These blocking/deblocking reactions will be useful for the introduction of inhibiting groups at specific positions on the polynucleotide "carrier" molecule. To date, a tetranucleotide has been prepared and characterized and the general scheme appears to be applicable to the synthesis of larger molecules.

Significance to Biomedical Research and the Program of the Institute: Within the past few years, there has accumulated an important body of data which show that in many different vertebrate cell types, most of the RNA that is made in the cell nucleus is destroyed in the nucleus itself very shortly after synthesis. The figures that have been published indicate that as much as 90% of the newly synthesized RNA is destroyed in the nucleus itself, and that its half-life may be only a few minutes.

This entire field is still virtually untouched with respect to the problem of carcinogenesis. It is well established that chemical carcinogens have immediate and profound effects on the metabolism of RNA in the cell nucleus. The development of a selective drug probe, which would enable the investigator to stop the destruction of messenger RNA in the cell nucleus (without interfering with its synthesis), would open up a wide variety of experimental work in the field of chemical or viral carcinogenesis, as well as studies in related fields such as enzyme induction or mechanisms of cell differentiation. Thus, a totally new approach will become available to study the mechanisms whereby chemical or viral carcinogens, as well as physiological stimuli such as steroid hormones, affect the metabolism of messenger RNA in the cell. It is apparent that there is a common interest in mechanisms of carcinogenesis in the Etiology Area, and it is thus hoped to provide a new research tool to investigators who would wish to use it.

In addition to the investigations described above, compounds being synthesized under this contract are presently being assayed for their possible inhibition of the RNA-dependent-DNA polymerase of oncogenic viruses and leukemic cells. These collaborative studies are being performed by Dr. George Todaro, Viral Oncology, Etiology, NCI, and by Dr. Robert Gallo, Chemotherapy, NCI. The relevance of these studies to viral carcinogenesis are of paramount importance.

In December 1970, the Project Officer organized a two-day workshop on "Penetration and Uptake of Nucleotides into Animal Cells", attended by over 20 guests, selected to include the leading scientists in the field. Scientific questions, originating from the goals of this contract, were largely responsible for the original decision to hold such as workshop, and its conclusions were an indication of high scientific interest in the pursuit of this project.

The contract is thus well integrated into the overall program of the Institute, serving to develop an important new area of synthetic organic chemistry, and as a source of new test materials for biological assays in different areas of NCI.

Proposed Course: To continue to develop new synthetic nucleotide analogs that will have greater biological potency and selectivity. To continue to test these nucleotide analogs in carcinogenesis test systems such as their effects on malignant transformation of cells by Rous sarcoma virus, as well as for their effects on the RNA-dependent-DNA polymerase of oncogenic viruses and transformed cells.

Date Contract Initiated: June 21, 1966

Current Annual Level: $127,000

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program - Research on Co-Carcinogenesis

Contractor's Project Director: Dr. James Liverman

Project Officer (NCI): Dr. Gio Gori

Objectives: The broad objectives are (A) to carry out large-scale co-carcinogenesis studies in exposure chambers with controlled atmospheres containing selected chemical agents to be tested for direct carcinogenic effects on respiratory tissues or for co-carcinogenic effects with known carcinogens, radiation, and viruses; (B) to bring to bear on problems of chemical and viral carcinogenesis the diverse scientific talents of the staff of the ORNL Biology Division and the scientific and engineering expertise of other ORNL components. A major part of the funding is associated with the first objective. Specific objectives are (1) to develop and define animal models for study of the pathogenesis of human lung cancer with emphasis on interaction of exogenous and endogenous factors, using inhalation exposures as well as other methods for respiratory carcinogenesis studies; (2) to obtain comparative data on mutagenic and carcinogenic responses to various chemicals in microbial systems and in mammalian cells; (3) to develop tools to permit detailed study of the role of the genes of mammalian somatic cells in transformation to malignancy by chemical or viral agents; (4) to analyze the mechanisms for repair of damage to DNA by chemicals or irradiation in mammalian cells and the role of such damage and repair in carcinogenesis; (5) to analyze the interaction of host immune mechanisms with chemical carcinogens and RNA tumor viruses; (6) to detect and characterize changes in DNA, RNA and protein synthesis in cells and tissues transformed by chemicals or by oncogenic viruses; (7) to analyze the mechanisms by which gene expression is regulated in mammalian cells, with special attention to regulation of expression of oncogenic viruses; (8) to characterize cellular changes during carcinogenesis by the Shope virus and its mutants and to develop the use of passenger viruses to transmit specific genetic information; (9) to investigate the relationship between embryogenesis and oncogenesis, especially as regards the role of C type viruses in both processes.

Major Findings: (1) *Respiratory Carcinogenesis:* The first large-scale inhalation study investigating co-carcinogenesis of various factors in mice was completed in FY 1971. The final phase involved the study of 2,200 mice, half of which were exposed to calcium chromate for their lifespan. Other co-factors studied were X-irradiation and PR8 (influenza)virus infection. Both calcium chromate-exposed and X-irradiated animals developed four times as many lung tumors (adenomas and adenocarcinomas) as control animals. The effects of these two agents were not additive. Influenza virus infection suppressed both spontaneous and induced lung tumor incidence. The calcium chromate caused marked pathological changes in all parts of the respiratory tract.

The second long-term inhalation experiment in which co-carcinogenic effects of air pollutants are being examined in hamsters is about 50% complete. Artificial smog and ferric oxide dust are being examined for co-carcinogenic effects on DEN-induced respiratory carcinogenesis in hamsters. Multiple tracheal papillomas have been found in many of the DEN-injected hamsters. Whether the artificial smog or ferric oxide dust exposure will alter the tumor response to DEN is not clear from the presently available data.

The co-carcinogenic effects of reactive gases which are common urban and occupational air pollutants, SO_2 and NO_2, have not been adequately examined in the past. Preparatory to undertaking such a study, rats and hamsters are being used in experiments to establish tolerable dose levels of SO_2 and NO_2 under chronic exposure conditions.

Previous inhalation experiments showed that calcium chromate increases lung tumor incidence in mice and causes widespread damage of respiratory tissues. In order to determine whether calcium chromate would have carcinogenic effects in the respiratory tract of hamsters (in which bronchogenic carcinoma can be induced) the response of hamsters to calcium chromate alone and in combination with low levels of the carcinogens DEN and methylcholanthrene is being examined.

In a collaborative experiment with Dr. W. Lijinsky of the Eppley Institute, the question of whether respiratory infections play a role in the incidence or type of lung cancer developing as a result of treatment with cyclic nitrosamines is being studied. The experiment includes germfree rats, specific pathogen-free rats purposely infected with mycoplasma and myxovirus (a combination known to cause squamous metaplasia in the respiratory tract), and rats with spontaneous respiratory infection. Also the possible role of vitamin A in altering qualitatively and quantitatively the tumor response is being analyzed.

A study of the role of immunocompetence in the lung and regional lymphatic tissues has shown that stimulation of the immune system with strong antigens (such as heterologous red blood cells) will double the number of lung tumors developing in response to urethane injection in BALB/c mice.

In an attempt to apply exfoliative cytological techniques to the early diagnosis of bronchogenic carcinomas, methods have been developed to obtain tracheal washings from rats and hamsters. Adequate numbers of cells from all parts of the respiratory tract are found in the specimens and the procedures can be repeated frequently in the same animal without difficulty. Studies are underway of progressive cytological changes occurring during the development of chemically-induced respiratory tract cancers.

It was found that intratracheal injection of large repeated doses (5 mg, 5 times) of methylcholanthrene into rats caused the development of large squamous carcinomas in the respiratory tree within 6 to 8 weeks after beginning treatment. This surprising finding prompted a comparable study of the effect of intratracheal administration of methylcholanthrene in two strains of mice (0.5 mg, 6 times). Squamous cell tumors developed here as well, in both strains, within a few months after beginning methylcholanthrene treatment. The carcinomas developed in both rats and mice could be serially transplanted and the transplanted tumors maintain their squamous characteristics. There are indications that the primary tumors may be of alveolar as well as of bronchial origin.

Studies of the role of respiratory infection in lung clearance mechanisms have shown that influenza virus infection more than doubled the time required to clear a neutron activated chromium dust.

New efforts were developed in FY 1971 comparing the metabolism of carcinogenic hydrocarbons in various strains of mice with particular reference to metabolism by the respiratory tissues. Also techniques for organ culture and cell culture of rat and mouse tracheas were established during this year. Preliminary studies have been made of the response of such cultures to a variety of carcinogens added *in vitro.*

In another program newly developed in FY 1971 a cannula was developed for bypassing the nasal cavities of laboratory rodents to permit deposition of inhaled particulates in the bronchial tree and in the lung. This device is being used to investigate the feasibility of such an approach to the bioassay of the carcinogenicity of cigarette smoke. Experiments are underway to explore the optimal conditions for use of this device in conjunction with smoking machines.

(2) *Mutagenicity of Carcinogens:* The principal aim of this activity is to determine whether there is any correlation between carcinogenic and mutagenic activity of chemicals, and whether this involves particular types of mutagenic activity. Mutagenicity of carcinogens is being evaluated with test systems capable of detecting all types of genetic damage, some of which can be characterized at the molecular level, and particular attention is paid to assaying mutagenicity of metabolic derivatives (proximal and ultimate carcinogens) as well as the parent compounds. Test systems employed are the ad-3 locus of two component heterokaryons of Neurospora, and hamster cells cultured *in vitro* where gene mutation can be studied at several loci. The latter system offers the possibility of studying carcinogenesis and mutagenesis in the same cell. This approach is beginning to show a high correlation between carcinogenic and mutagenic activity, and also between carcinogenicity and a particular type of mutagenic activity. In general, it can be said that a high percentage of the mutations caused by carcinogens are those that would produce a gene product of low activity rather than the partial or complete absence of the gene product.

A variety of types of precarcinogens, proximate, and ultimate carcinogens were tested in the Neurospora system in FY 1971. Genetic characterization of the resultant mutants is not complete. The types of compounds tested include ethylene imines, naphthylamines, N-alkyltriazenes, quinoline oxides, aflatoxins, epoxides and acetylaminofluorene compounds. A high percentage of the mutants induced by aflatoxins and AAF compounds are those resulting from missense mutation; very few chromosome breaks were induced.

Development of methods for the production of ultimate carcinogens in the mutation assays has continued. These include chemical hydroxylation systems, microsomal enzymes, and host-mediated assays, and are being applied in mutation assays with Neurospora, hamster cells, and with Salmonella. In the latter system the carcinogen DMN was highly mutagenic under conditions favoring chemical hydroxylation but not when hydroxylation was prevented.

Carcinogenicity of DEN in Neurospora has been similarly examined with comparable results.

A host-mediated assay system for mutagenicity testing with Chinese hamster cells has been developed. In preliminary experiments it was found that there is a significant increase of spontaneous mutation frequency in cells sequestered in the abdominal cavity of a mammalian host. Similar results were found previously with Neurospora treated in this fashion.

A major effort was made to develop new assays for detection of gene mutations in Chinese hamster cells, preparatory to a planned effort to utilize these cells to develop a system in which *in vitro* carcinogenesis can be analyzed in cells of known genetic background and wherein the genes can be experimentally manipulated.

(3) *Somatic Cell Genetics:* A major barrier in understanding gene expression in mammalian cells, and thereby cancer etiology, has been the inability to manipulate specific segments of the genome by inducing mutations at known sites. The goal of this activity is to increase the genetic variability of human and other mammalian cells *in vitro* and to characterize the mutations with respect to their mode of inheritance, cytological mapping, linkage, etc. Attempts are being made to develop cell to cell transfer of genetic material through cell fusion, DNA transformation, or viral transduction. It has long been desirable to have an experimental system in which carcinogenesis and mutagenesis can be analyzed in the same cell. To further this objective, this program will be expanded in FY 1972 to include a correlated effort on chemical carcinogenesis of mammalian cells *in vitro*.

In FY 1971 selective techniques for isolation and identification of mutants in mammalian cell cultures were refined. The induction of forward and reverse mutations at the *azg* locus (controlling the activity of purine-phosphoribosyl transferase) has been made more precise through effective elimination of leaky mutations. Additional mutations at other loci, controlling activities of other enzymes involved in nucleic acid biosynthesis, have been obtained. A group of mutants which are resistant to cytosine arabinoside was isolated. Results indicate that there may be more than one locus that controls sensitivity to this analog. Spontaneous and induced mutations to temperature sensitivity were obtained in sublines of Chinese hamster cells. Dose-effect relationships with chemical mutagens such as EMS were developed and the *azg* mutants derived from X-irradiated cells were characterized genetically. Preliminary data indicate that both point mutations and chromosome deletions may have occurred in response to ionizing radiation.

During FY 1972 this program will be expanded to include a correlated effort on *in vitro* chemical carcinogenesis. The relationship of somatic mutation to malignant transformation *in vitro* will be a primary goal of this new program. Additionally it may be possible, ultimately, to relate expression of specific genes to the malignant transformation.

(4) *Repair Mechanisms in Carcinogenesis:* This activity employs the techniques of UV photobiology and DNA isolation, hybridization, and sedimentation to analyze the repair of UV and chemical damage in mammalian cells *in vitro* and *in vivo*. The objectives include understanding of mammalian repair systems in general and specifically (a) to correlate the absence of well-coordinated repair with human disease, (b) to determine if repair systems work on chemical damage to DNA, and (c) to determine if UV-induced pyrimidine dimers can give rise to skin cancer.

A new and sensitive assay for excision repair of DNA has been developed; this assay yields the numbers, sizes and distribution of the repaired regions. Repair is permitted to take place in the presence of BUdR and the cells are then irradiated with a large flux of 313 nm radiation resulting in breakage of the patched regions when the cells are lysed and the DNA sedimented in alkali. The method is applicable to all types of repaired lesions. It has been used to show that fibroblasts from the hereditable skin cancer Xeroderma pigmentosum have a repair capacity which is ten-fold smaller than in normal fibroblasts. Also repair after UV damage is easily observed in cells derived by transabdominal amniocentesis and therefore the method may be used as a prenatal assay for potential Xeroderma pigmentosum. Previous studies on cells from patients with this disease have been extended to six new patients; the results show that there is no functional "UV endonuclease" in any of these cells.

In cultured cells of submammalian species (which unlike mammalian cells, have photoreactivation capacity) mitochondrial and nuclear DNAs were found to be photoreactivated at nearly identical rates. This establishes that the repair mechanisms for these two cellular DNA types are comparable.

In a collaborative effort with Drs. J. K. Setlow (ORNL Biology Division) and P. O. Tso (Johns Hopkins University) it was shown that transforming bacterial DNA, treated chemically with benzpyrene, can be partially reactivated by bacteria able to repair UV-damaged DNA. Mutant bacteria lacking the latter capacity are also deficient in repairing chemical damage to DNA.

Normal skin fibroblasts and skin fibroblasts from a Xeroderma patient have been exposed to low levels of UV irradiation for periods of several weeks, and the morphology of the resultant cultures studied. Most of the normal cells survived and retained their typical fibroblast morphology. However, in the irradiated Xeroderma cultures, patches of cell developed which had some characteristics of neoplastic transformation, such as multinucleated cells, increased basophilia, loss of contact inhibition, etc. This suggests a necessary relationship between UV-induced pyrimidine dimers in DNA, the lack of capacity to repair these dimers, and the subsequent development of skin cancers.

(5) *Immunology of Carcinogenesis:* During FY 1971 this activity developed two discrete but related research programs, one concerned primarily with viral oncology and the other primarily with chemical carcinogenesis.

The major goal of the first of these is to study the interaction of RNA tumor viruses with the host immune mechanisms. The major questions being asked are (a) What is the basis for selective or preferential virogene expression in immunocompetent cells? (b) What are the essential relationships between RNA tumor virus-mediated pathogenesis and systemic immune suppression of the host? (c) During some period of development of the host animal does an intrinsic balanced immunity exist with respect to endogenous RNA-tumor virus? (d) Does virogene or oncogene activity play a role in normal immunocompetent cell function? The major approach utilized is to correlate the conventional cellular and serologic parameters of immunity with histologic and ultrastructural changes of the target tissue. These and other parameters are used to evaluate suppression and recovery of immune potential from attack by viral agents as well as alterations of immune status in germfree, conventional, aging and thymectomized animals. Within the scope of this research program is investigation of other sources of antigens which crossreact with specific transplantation antigens of RNA tumor virus-infected cells.

Early histologic and ultrastructural changes in lymphatic tissue were studied in adult BALB/c mice given Rauscher virus (RLV). It was found that (a) endogenous C-type virus is localized as antigen in antigen-retaining reticular cells of germinal centers, (b) it is possible that some forms of virus destruction occurs in these extracellular regions, (c) virus replicates in the parenchymal immunoblast compartment of the germinal centers, (d) amplification of this compartment by other antigens enhances RLV spenomegaly, (e) maturation arrest in the immunocompetent cell compartment correlates with RLV-induced immune suppression, (f) viropexis of C-type virus between immunoblast and hematopoietic cells of the spleen was found. In these experiments it was also found that there may be a synergistic effect of passenger lactic dehydrogenase virus (LDV) with the RLV virus in causing the pathology associated with "Rauscher disease". LDV was found to replicate in the thymic parenchyma 48 hours after injection directly into the thymus. This is the first pathology associated with LDV. Results strongly imply a synergistic interaction between this passenger and the Rauscher virus. This question was examined further in the Viral Carcinogenesis Group of this Program and will be discussed below.

In a collaborative study with J. H. Coggin of the MAN Program of ORNL, it was found that humoral antibodies, generated as a result of immunization with isologous fetal tissue, depress the growth of RLV-infected spleen cells and plasma cell tumors in BALB/c mice. The data suggests the presence of an embryonic antigen which crossreacts with neoantigen of both the RNA virus-infected spleen cells and the plasma cell ascites tumor. These embryonic antigens can immunize adult animals, making them capable of suppressing RNA virus-induced tumor growth and pathogenesis. Also adoptive transfer of fetal antigen-primed spleen cells can suppress RLV-induced splenomegaly.

RFM mice have a low spontaneous leukemia incidence but are quite susceptible to radiation-induced leukemia. In these mice antigens of RNA tumor viruses can be detected in the thymus after two weeks of age and reach a peak at seven weeks of age. Detection of the virus in the spleen occurs between 4 and 5 weeks of age followed by a marked drop at 10 weeks. The appearance of virus in the spleen correlates with the development of immune competence and capacity to trap antigen. These results are interpreted as an immune development to the virus and can be correlated with the development of glomerulosclerosis which occurs in these mice after 20 weeks of age; virus antigen and antibody can be detected in the kidney during this interval. When C3H/Bif mice become immunologically mature they lose susceptibility to Gross virus-induced thymoma. This susceptibility is restored after low levels of irradiation. The incidence of thymomas in irradiated adult C3H/Bif mice is markedly suppressed if the animals are injected with newborn thymus cells or with the Gross virus. These results clearly demonstrate that immune suppression is a factor in susceptibility of C3H/Bif mice to Gross virus-induced thymoma.

Germfree mice, unlike their conventional counterparts, do not develop plasma cell tumors after mineral oil injection. An experiment is underway to determine if this inability to develop plasma cell tumors is a result of the absence of target cells in Peyer's patches. This is being examined in germfree animals, some of which have antigen-stimulated Peyer's patches. Preliminary results suggest the development of plasma cells after such stimulation, but it is too early to determine whether or not tumor formation will be observed as a result of this stimulation.

A study was completed in AKR mice which demonstrated that the typical preleukemic changes found ordinarily in older mice, can be induced in 3-month-old animals by intrathymic injection of Gross virus. Experiments are planned to immunize with Gross virus vaccine to determine if the development of the immune mechanism in the thymus can render some degree of protection to spontaneous thymoma incidence.

An investigation was completed of the relation between the development of reticulum cell sarcoma and the reduction of immune capacity in aged $BC3F_1$ mice. It was found that the decrease in immune capacity in aged mice is clearly not a function of the development of lymphoreticular malignancy. The results suggest, however, that impaired immune competence may be related causally to the subsequent development of lymphatic and non-lymphatic tumors and that "mesenteric lymph node disease" is a degenerative disease associated with antigen burden.

In the area of chemical carcinogenesis a new program was developed in histopathologic and functional aspects of systemic tumor immunology. The aim of these studies is to correlate both the histopathologic aspects of the systemic immune response and anatomic factors which involve the progressive growth of chemically-induced primary and secondary tumors with functional immunologic factors directed against success of the tumors. Studies are being carried out in three host-tumor systems. One of these is DMBA-induced carcinomas in hamsters; a second is SV40-induced sarcomas in hamsters; and the third is syngeneic hepatoma cell transplants in guinea pigs. It is hoped that by studying these three very different animal tumor models, evidence can be obtained regarding (a) cooperative or antagonistic interactions between various components of the immune system and the anatomic and physiologic limitations within the systemic immune response which limit the ability of the host to focus immunity on the tumor, (b) the anatomic limitation at the site of tumor growth, (c) the involvement of cytotoxic immunity and humoral immunity, (d) factors in serum which can block or mask immunity, and (e) the mechanism of tumor regression mediated by such immune stimulants as BCG.

(b) *Enzymology of Carcinogenesis:* The long range goal of this activity is an understanding of the biochemical mechanisms involved in the initiation of cancer, i.e., to detect and characterize the heritable changes in macromolecular synthesis which develop in the course of chemical and viral carcinogenesis. Translational mechanisms, particularly those involving transfer RNAs, have been of particular interest in this work. Current objectives include (a) establishment of a cell culture system amenable to viral transformation with cell lines having different susceptibilities to the action of oncogenic viruses, (b) a study of the initial biochemical events involved in transformation by these viruses, (c) biochemical analysis of the host factors acting during chemical and viral carcinogenesis, (d) isolation and characterization of the macromolecules synthesized in cells undergoing viral and/or chemical carcinogenesis. Emphasis is on biochemical technology developed at ORNL for microanalysis of translational mechanisms in protein synthesis.

In FY 1971 a study of changes in isoaccepting tRNAs in tumor and embryonic tissues was completed. A survey of tyrosyl- and aspartyl-tRNAs was carried out in 6 transplantable tumors, 5 spontaneously occurring primary tumors and 3 cultured tumor cell lines. All of these tumors showed two types of aspartyl and tyrosyl tRNAs: the normal differentiated cell type, and the abnormal "stem" cell type, in various proportions according to the individual tumor. The abundance of the abnormal "stem" cell type appeared to be proportional to the malignancy of the tumor. These abnormal tRNAs are not, however, unique to tumor tissue; they are also detected in embryonic tissues.

The role of aging in oncogenesis has been a concern of this group for sometime. A study completed in FY 1971 involved biochemical tests of the "error catastrophe" theory of Orgel, which postulates that errors involved in expression of genetic information during translation increase in magnitude as the animal ages. Comparison of the isoaccepting tRNAs of the liver and brain between mature and aged BC_3F_1 mice was made by the chromatographic systems developed at ORNL; no significant differences were found. Similarly no differences were detected in the aminoacyl-tRNA synthetases in these tissues.

During FY 1971 a study was initiated of the host factors acting to regulate expression of oncogenic viruses. In initial work the RNA dependent DNA polymerase was used as an indicator of virus expression. Template requirements, metal ion requirements, and other parameters of polymerase activity in extracts of purified RLV were carefully investigated to determine the optimal assay conditions. The results of these studies suggest that chain initiation is rate limiting for this polymerase. Normal and RLV infected mouse spleens were fractionated and the fractions tested for capacity to inhibit or activate added RLV polymerase. Several such components were found. A major inhibitory component of the normal spleen is not present in the RLV-infected spleen. These preliminary results suggest that there may, indeed, be cellular host factors acting to regulate virus expression via controlling the RNA-dependent synthesis of DNA. These studies will be continued in FY 1972 and expanded to include comparable studies of cells transformed by chemical carcinogens.

(7) *Regulation of Gene Expression:* Carcinogenic agents apparently act by freeing from restraint many genes that are normally kept repressed within differentiated tissue. Evidence for such derepression is found in the appearance of fetal and viral associated antigens in differentiated cells transformed by carcinogens. This activity concentrates on the molecular mechanisms operating to regulate expression of specific genes in mammalian cells. As the products of gene expression, the cellular enzymes, can be regulated in amount by degradative as well as synthetic processes; both of these are being studied. Hepatoma cells (H35) growing in culture provide the focus for much of this work. During FY 1971 a new effort was initiated wherein techniques and concepts developed in previous research on hormonal regulation of gene expression, are being applied to the mechanisms regulating expression of oncogenic virus components.

Work on the model regulatory system, tyrosine transaminase induction in H35 cells, continued in FY 1971 with emphasis on the mechanism by which steroid hormones effect the release of transcriptional repression to permit accelerated synthesis of specific mRNA. Specific steroid binding proteins were partially purified and results obtained which support the conclusion that binding to nuclear receptors is a necessary prerequisite to enzyme induction. Preliminary fractionations indicate that the receptor is located in chromatin. The chromatin from steroid-treated cells has increased capacity to serve as template for synthesis of RNA. Synchronized cells were found to be incapable of responding to the steroid inducer during both G2 and M phases of the cell cycle. It was found that transaminase synthesis and degradation are both changed by high concentrations of L-leucine. This effect is independent of hormone action; preliminary evidence for a leucyl-tRNA species which requires high concentrations of leucine for charging will be examined further.

The enzyme alanine transaminase, known to be inducible by steroid treatment *in vivo*, was found to respond to hydrocortisone addition to cultured H35 cells in a comparable fashion. Differences between the response of this enzyme and that of tyrosine transaminase suggest that there may be sequential rather than coordinate effects of the steroid on transcription processes. Factors regulating the activity of ornithine decarboxylase, an enzyme which undergoes large increases in cells and tissues stimulated to rapid growth, were examined in rat liver. A number of hormones were tested for effects on activity levels of this enzyme; results indicate that none of the hormones tested is acting directly to induce ornithine decarboxylase.

The concept that physical properties of an enzyme molecule are of primary significance in determining its rate of degradation was analyzed by changing the properties of tyrosine transaminase in cultured cells. Enzyme induced in medium containing 5-fluorotryptophan was three times more heat labile and only 63% as active as the native enzyme but was degraded *in vivo* at the normal rate. Thus it would appear that the physical properties are not of primary significance in degradation. A cell-free system in which it appears that cellular processes of degradation can be analyzed has been developed. Gently-lysed suspensions of H35 cells degrade added enzymes at rates comparable to the degradative rates for these enzymes in the cell.

Studies of regulation of virus expression were initiated during FY 1971 with concentration of two immediate objectives (a) development of an experimental system in which expression of oncogenic viruses can be observed and can be manipulated experimentally and (b) development of a sensitive, precise and specific assay for virus expression. Most of the initial effort was toward the latter goal where it was asked whether the viral type RNA-dependent polymerase might serve as an appropriate marker. In future work polymerase assays will be correlated to serologic assays for gs antigen activity. It is hoped that the sensitivity of the enzyme assay will be complemented by the specificity of immunological assay. Initial efforts were made toward purification of the various internal protein components of the Rauscher leukemia virus. These will be fractionated to homogeneity and used to prepare specific antisera for future work. Initial efforts were made to correlate these assays for virus expression in two experimental systems (a) during fetal development, and (b) in the estrogen-stimulated endometrium of ovariectomized mice. When the objectives of valid assay and a convenient experimental system have been met, analyses of the molecular mechanisms involved in regulation of expression of virus genes will begin.

(8) *Biochemistry of Viral Carcinogenesis:* This activity has two major goals which are roughly interrelated (a) studies of the mechanism of action of the Shope papilloma virus in inducing papillomas in the epithelium of rabbits and its relation to the resulting cancers and (b) the use of viruses as vectors of genetic information to correct deficiencies and to modify the enzymatic state of the host as a therapeutic measure.

Comparative studies of the physical and chemical characteristics of wild-type REC mutants of the Shope virus are continuing. Unlike the wild-type virus, the REC mutant has no detectable capsomers. Arginases induced by the two virus lines are separable; amino acid sequences of purified arginase from each source are now being determined.

Two cancers arising from the virus-induced papillomas of rabbits have been transplanted over a period of many years. The Vx-7 cancer still has virus and the virus induced arginase after 115 transplant generations. The Vx-2 cancer has lost both virus and arginase. Each has specific and unusual amino acid requirements which may lead to metabolic control of these cancers using specific antagonists and dialysis against specific enzymes. Upon reintroduction of the virus into Vx-2 amino acid requirements and compositions change to resemble closely the Vx-7.

Specifically hybridized Shope virus messenger RNA has been isolated from papillomas and separated into 5 fractions by equilibrium density centrifugation. The capacity of these RNAs to code for arginase or other virus proteins is being examined using an *in vitro* ribosomal system capable of appreciable and precise protein synthesis.

Cultured fibroblasts from children suffering from arginase deficiency disease have been inoculated with the Shope virus. Preliminary results indicate some induction of arginase in such cells.

Studies are being made of methods for coating virus RNA and DNA to improve the capacity of isolated nucleic acids to infect cells. This will be a necessary step in future developments where it is planned to chemically or enzymatically modify such viral nucleic acids. Polymers of basic amino acids (e.g. polylysine), when used to coat viral nucleic acid, will improve resistance to nucleases but also lowers infectivity titer; apparently the infected cell has difficulty removing this type of a synthetic protein coat.

(9) *Viral Carcinogenesis:* This activity has two major components, one concerned with RNA tumor viruses and developmental processes, and the other with the nature of the interactions between cells, viruses, and carcinogens.

Research efforts in the first component are aimed toward the commonality between embryogenesis and oncogenesis and its possible significance in the control of cancer. Alteration in the isoenzyme patterns of a variety of tumor types and corresponding normal and fetal tissues was completed in FY 1971. In future work this will be correlated with the covert expression of virus antigens. Isoenzyme changes in lung and stomach tumors induced by chemical carcinogens reveal changes identical to those seen in leukemic tissues resulting from RNA virus infection. Like gs antigen expression, the specific isoenzyme patterns associated with these malignancies are also evident in fetal tissues.

Analogies between fetal and malignant tissues prompted an investigation of the degree and specificity of ^{67}Gallium (Ga) localization during embryogenesis. Isotope uptake in embryos was determined directly and by autoradiography. About midway during gestation, ^{67}Ga uptake in embryonic tissues was equivalent to or surpassed that previously detected in leukemic tissues of adult mice. Uptake of ^{3}H-thymidine was not comparable to that of ^{67}Ga, demonstrating that gallium uptake is not a reflection of cell turnover.

An examination of spleen weight, histology, and isoenzyme profiles in pregnant mice showed that these parameters changed during pregnancy in a fashion parallel to that previously determined in LDV infection. Serum proteins are also altered similarly in the two conditions. Preliminary results suggest a modest reduction in splenomegaly following RLV-infection in multiparous mice compared to virgin animals. Similarly, preliminary results indicate that multiparity may have a suppressive effect on the expression of spontaneous leukemia in RFM mice.

A study completed in FY 1971 showed clearly that the syndrome resulting from infection with RLV is almost entirely dependent upon the presence of lactic dehydrogenase virus (LDV). Mice treated with RLV preparations contaminated with LDV showed early growth and histological changes in their lymphatic tissues which were almost identical to those in mice inoculated with LDV alone. Cell culture-derived RLV, not contaminated with LDV, did not cause the changes associated with the "Rauscher disease" syndrome, except the early erythroblast activation. The clean RLV preparations fail to cause lymphocyte destruction in thymus-dependent areas responsible for cell mediated immunity, as well as immunoblast activation and proliferation in lymphoid germinal centers responsible for the humoral immune response. Intentional contamination of RLV with LDV resulted in an enhancement of the erythroblastic reaction associated with "Rauscher disease" as measured by spleen weight increase. There were also prominent changes in thymus-dependent areas and germinal centers of lymphatic tissues when the virus mixture was employed. Thus many of the properties associated with the RLV syndrome, especially immunosuppressive reactions, appear clearly to be due to contaminant LDV.

Progress in the second area included demonstration of *in vitro* cytotoxicity of fetal antigen-primed spleen cells, against cells infected with Moloney leukemia virus. The irradiated fetal tissues used for immunization in these studies were negative for leukemia virus antigens by several tests. The spleen cells from immunized mice were found to incorporate thymidine upon exposure to thymidine labeled target cells suggesting a true immune reaction. These results suggest that cells infected with MLV possess antigens similar to those of certain isologous embryonal cells.

Effects of fusion of permissive and nonpermissive cells on leukemia virus synthesis have been examined. This approach is based on the premise that susceptibility may be regulated intracellularly. Techniques have been developed for simultaneous autoradiography and fluorescence microscopy, and cell fusion. Preliminary results are suggestive that incorporation of a nucleus from a human cell reduces the capacity of the recipient cell for virus synthesis.

In collaborative studies with the Immunology Group of the Carcinogenesis Area, evidence was obtained that the basis for adult resistance to Gross virus in C_3H/Bif mice is an immunological phenomenon. Further, adoptive transfer of newborn (but not adult) isogenic thymic cells protected against the virus induced thymoma, indicating both that immune function is involved and that an age-dependent target cell population does not exist for Gross virus. Additional studies with RFM mice have indicated that a normal immune response may exist for endogenous leukemia virus. The appearance of virus antigen in the spleens of RFM mice correlates with the acquisition of immunocompetence, and the virus reappears in the spleen after the irradiation. Experiments are in progress to determine if the radiosensitive component of the immune system can be restored by adoptive transfer of isogenic cells from mice immunized against virus or fetal antigens.

Initial steps were taken in FY 1971 to mount a major effort toward the preparation of specific reagents and tests to detect covert virus expression.

Significance to Biomedical Research and the Program of the Institute: Bioassay of carcinogenic and co-carcinogenic effects of inhaled atmospheric contaminants and the development of suitable experimental models to assess the interaction of exogenous agents with endogenous factors in development of lung cancers are major goals of the Carcinogenesis component of the NCI Etiology Program. Supporting research in genetics, immunology, biophysics, and biochemistry on such questions as the relationship between mutagenesis and carcinogenesis, repair of chemical and irradiation damage to DNA, the changes in macromolecular synthesis in cells transformed by chemicals or viruses, and the interaction of host immune mechanisms with carcinogen or virus induced cancers, is considered to be essential to the long range goals of the entire program. The concerted interdisciplinary effort involving immunologists, biochemists and virologists being developed in viral carcinogenesis and approaching key questions such as mechanisms of regulation of expression of oncogenic viruses and the relationship of embryogenesis and viral oncogenesis is of particular significance to the Viral Oncology component of the program. Many of these studies are correlated with ongoing research in radiological aspects of cancer causation at ORNL; this program thus accomplishes a desirable integration of NCI efforts in chemical and viral carcinogenesis with AEC programs in radiation carcinogenesis.

Proposed Course: Continuation of the program aimed at attaining the objectives described above is proposed. Research on carcinogenic effects of inhaled materials will be expanded to include development of bioassay systems for carcinogenicity of cigarette smoke. Studies in somatic cell genetics will be correlated with *in vitro* carcinogenesis to develop both the relationship between mutagenesis and carcinogenesis and the role of cellular genes in malignant transformation. New emphasis will be placed on biochemical analyses of host factors determining susceptibility to chemical and viral carcinogens and of cellular mechanisms governing expression of oncogenic viruses. The relationship of fetal antigens to virus-induced cancers and the role of host immune mechanisms in determining response to chemical carcinogens, studies which were initiated in FY 1971, will continue in FY 1972. Work relating known lesions in DNA to transformation and malignancy will be given increased emphasis.

Effective with FY 1972, the responsibility for funding of this Program has been assumed jointly by the Carcinogenesis and Viral Oncology Areas of Etiology, NCI. The research projects have been assigned to the area of prevalent relevance. Monitoring and reviewing will be conducted by the two Areas for their respective programs, with appropriate liaison.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,800,000

BATTELLE-NORTHWEST LABS., INC. (PH43-68-1372)

Title: Inhalation Co-Carcinogenicity of Industrial Pollutants and Cigarette Smoke

Contractor's Project Director: Dr. Alfred Wehner

Project Officer (NCI): Mr. Raymond Newell

Objectives: The project is devoted to a long-term inhalation bioassay of combined exposures to cigarette smoke and a dust (crysotile asbestos, cobaltous oxide or nickelous oxide). Syrian golden hamsters are exposed to fresh cigarette smoke in a Hamburg-type 2 machine and to whole body dust exposure alternating with the smoke exposures. The dust distribution and characterization within the chambers are being obtained. Analysis of inhaled chemicals in tissues is performed at intervals by suitable techniques (atomic absorption spectrophotometry, neutron activation, etc.). Detailed pathological studies are conducted in all animals.

Major Findings: The long-term tests are under way. An improved asbestos disseminator has been developed. Tests have been conducted to determine the lung retention of the dusts after inhalation exposures and the translocation of the three materials from the gastro-intestinal tract to the liver and kidney.

Significance to Biomedical Research and the Program of the Institute: This project is the only one in the Carcinogenesis Program, designed to test the combined effects of exposures to cigarette smoke and environmental dusts. The three dusts were selected on the basis of previous studies by direct administration of the single materials in the respiratory tract.

Proposed Course: The experiment is now in its chronic exposure phase. Exposures will continue into the summer of 1972. Final evaluation of the effects and compilation of the data will require about six months so that the entire project will take approximately two more years.

Date Contract Initiated: June 28, 1968

Current Annual Level: $169,000

BIONETICS RESEARCH LABORATORIES (NIH-69-2085)

Title: Carcinogenicity of Chemicals Present in Man's Environment

Contractor's Project Director: Dr. B. M. Ulland

Project Officer (NCI): Dr. John Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity in rats of a number of chemicals used in commerce and industry. Also to investigate the possible teratogenic effects of these environmental agents.

Major Findings: Thus far, approximately 70% of the animals treated with industrial chemicals, pesticides and other environmental agents have come to autopsy. The remainder of the groups will continue for another year. Detailed histological study of tissues obtained from the animals autopsied are now in progress. A number of the agents studied were carcinogenic, some highly so. Propyleneimine and propane sultone, proposed for use or used in industry were highly active and interestingly lead to the induction of brain tumors. There are relatively few compounds that give high yields of brain tumors quickly. Thus, these agents may find application in fundamental studies dealing with this problem. At the same time, a note is in press to alert the scientific and technical public about hazards attached to these compounds. The fungicide, ethylenethiourea, which caused liver tumors in mice in the previous Bionetics pesticide study (Innes, *et al: J. Nat. Cancer Inst.* 42: 1101, 1969), in the present series induced tumors of the thyroid in rats. The antioxidant butylated hydroxytoluene (BHT), but not the antioxidant diphenyl-para-phenylenediamine (DPPD) has reduced tumor induction by a known carcinogen, used as positive control compound.

Significance to Biomedical Research and the Program of the Institute: One primary effort in the prevention of cancer consists in determining potential carcinogenic hazards in the environment. The aim of this program is to gather such data for a number of widely used materials. Additionally, the test procedures and the results obtained will be examined critically with the aim of improving the methodology by which such assays are conducted. Information on the teratogenic properties of these same chemicals will likewise assist the areas of preventive medicine, and simultaneously yield data of significance with respect to the problem whether the carcinogenic and teratogenic properties are related.

Proposed Course: The last group of animals on test, compounds including the important groups on saccharin, will come to autopsy in the summer of 1972. It is expected that the main portion of the contemplated programs will terminate by the end of 1972, with the exception possibly of teratology and transplacental carcinogenicity studies.

Date Contract Initiated: June 28, 1968

Current Annual Level: $150,814

BIO-RESEARCH CONSULTANTS, INC. (PH43-68-1311)

Title: Carcinogenicity of Chemicals Present in Man's Environment

Contractor's Project Director: Dr. Freddy Homburger

Project Officer (NCI): Dr. John Weisburger

Objectives: The long-term effect of chronic administration of a number of chemicals to mice and rats is being evaluated.

Major Findings: The main groups of mice and rats phased in systemically with different compounds and drugs beginning in the fall of 1968 will reach the planned termination point and come to autopsy this Fall. In some instances, the carcinogenicity of a compound has led to an earlier conclusion. These groups include mice and rats treated with the widely used industrial compounds o-toluidine and 4-chloro-o-toluidine, which led to hemangiosarcomas, breast tumors, and a number of other cancers. Also active was 2,5-dimethyloxy-4'-aminostilbene, advertised as an intermediate for the production of industrial chemicals.

Significance to Biomedical Research and the Program of the Institute: One primary effort in the prevention of cancer consists in determining potential carcinogenic hazards in the environment. The aim of this program is to gather such data for a number of widely used materials. Additionally, the test procedures and the results obtained will be examined critically with the aim of improving the methodology by which such assays are conducted. Information on the teratogenic properties of these same chemicals will likewise assist the areas of preventive medicine, and simultaneously yield data of significance with respect to the problem whether the carcinogenic and teratogenic properties are related.

Proposed Course: Autopsies of experimental and control groups leading to data generation will begin this Fall and extend over approximately one year. It is expected that the overall program would be completed by the end of 1972.

Date Contract Initiated: June 26, 1968

Current Annual Level: $169,058

UNIVERSITY OF CALIFORNIA AT SAN DIEGO (NIH-70-2206)

Title: Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay

Contractor's Project Director: Dr. Michael Shimkin
Dr. Alex Kniazeff

Project Officer (NCI): Dr. Gio Gori

Objectives: Testing of chemicals for carcinogenic activity by induction of pulmonary tumors in strain A mice. Compounds to be tested include food additives from the GRAS list, drugs, pesticides, nitrosamines, carbamates and related compounds, and crude materials such as tobacco tars. An exploratory portion of this activity will study competitive carcinogenesis situations. Also the role of total length of exposure versus total doses will be studied. Finally, SPF animals will be treated with carcinogens and viruses in germ-free conditions, to establish co-carcinogenicity effects.

A distinct part of the project will evaluate lung organ cultures for conducting *in vitro* carcinogenesis tests including various transforming agents and viruses.

Major Findings: In the first year of operation the contractor has demonstrated the feasibility of the bioassay system proposed, and the desirability of continuing expanded developmental work that may lead to an *in vitro* carcinogenesis bioassay using organ cultures.

Significance to Biomedical Research and the Program of the Institute: A specific goal of the Carcinogenesis Area is to develop rapid bioassay methods to insure timely evaluation of the carcinogenicity of the countless compounds that have environmental significance. This contract is part of the effort to reach this goal. In view of the accomplishments done in the first year of operation, the contract will both provide an extension of existing bioassay capabilities and provide developmental research for improved and fast testing of methodologies.

Proposed Course: To continue as outlined above.

Date Contract Initiated: June 22, 1970

Current Annual Level: $104,720

UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO (NIH-70-2218)

Title: Correlation of Effects of Chemical Carcinogens in Human, Primate, and Hamster Epithelium *In Vitro*

Contractor's Project Director: Dr. T. Timothy Crocker

Project Officers (NCI): Dr. Michael Sporn
Dr. Umberto Saffiotti

Objectives: The objectives of this contract are to determine the following: (1) Do specific chemical carcinogens (which are known to cause bronchogenic carcinoma in animals) cause the same sort of preneoplastic changes in the normal respiratory epithelium of man that they are known to cause in the respiratory epithelium of animals? (2) Can the anti-carcinogenic effect of vitamin A on respiratory epithelium, which has been demonstrated in hamsters (in long term experiments *in vivo* and in organ culture *in vitro*), be meaningfully reproduced and defined in the respiratory epithelium of rodents, primates as well as of man?

The specific experimental tool which this project uses is the tracheobronchial organ culture system which Crocker and his colleagues have developed over the past five years. They are attempting to determine the following: (1) Do respiratory carcinogens such as benzpyrene and other polynuclear hydrocarbons, nitrosomethylurethane, or others, when applied to explants of human trachea or bronchus, cause the same development of squamous metaplasia, cellular pleomorphism, atypia, or other pre-neoplastic changes, that they cause when applied to explants of trachea of bronchus from rodents or primates? (2) Does the administration of vitamin A to organ cultures of tracheobronchial epithelium, either before, during, or after the administration of chemical carcinogens such as benzpyrene, nitrosomethylurethane, or nitrosamines, block the development of preneoplastic changes caused by these carcinogens? Is this effect similar in animals and in man?

Major Findings: Vitamin A blocks the neoplastic effect of benzpyrene on isolated tracheal epithelium. However, it does not alter microsomal metabolism of benzpyrene suggesting that the effect of vitamin A may be a more fundamental one on differentiation. Vitamin A has also been found to block the neoplastic effect of diethylnitrosamine (DEN) on isolated tracheal epithelium. It has been difficult to obtain enough human tracheal material for complete studies, but correlations are beginning to appear between the human and animal studies.

Significance to Biomedical Research and the Program of the Institute: It is now established that polycyclic aromatic hydrocarbons such as benzpyrene can cause bronchogenic epidermoid carcinoma in rodents (such as hamsters). The contractor has recently observed the same effect in a subsimian primate, the bushbaby, *Galago crassicaudatus*. Moreover, epidemiological evidence strongly incriminates polycyclic aromatic hydrocarbons, together with other compounds found in tobacco smoke, as etiologic agents for bronchogenic carcinoma in man.

The tracheobronchial organ culture system developed by Dr. Crocker offers an excellent opportunity to study the direct effects of such chemical carcinogens on respiratory epithelium, and a unique opportunity to correlate the effects of chemical carcinogens on the respiratory epithelium of both animals and man. Thus, tracheobronchial organ culture is currently the only available experimental system in which one can investigate the question whether specific chemical carcinogens cause the same type of preneoplastic changes in the normal respiratory epithelium of man that they are known to cause in the respiratory epithelium of experimental animals. If the answer is affirmative, then the relationship of many of the critical animal studies in the entire Carcinogenesis Program for human lung cancer will be even more important, in an immediately apparent manner. The ability to correlate studies in experimental animal pathology with human lung cancer is of utmost importance in the total program, and the current project is a direct attempt to obtain hard data in this area.

In addition, this project represents a first attempt to deal with the problem of anti-carcinogenesis using an *in vitro* test system. Although inhibition of respiratory carcinogenesis by vitamin A in the intact animal has been established, the biochemistry of the phenomenon is not known. Our ability to deal with the problem of anti-carcinogenesis in the future certainly will depend to a large extent on how much we understand about its biochemistry. Definition of the parameters which affect the ability of vitamin A to reverse benzpyrene or other chemical carcinogenesis should be of great future practical use in the entire carcinogenesis program.

Proposed Course: To continue organ culture studies correlating the effects of carcinogens on human tracheas and tracheas of experimental animals. To continue to study the anticarcinogenic effects of vitamin A on tracheobronchial epithelium of man and experimental animals.

Date Contract Initiated: June 23, 1970

Current Annual Level: $100,000

UNIVERSITY OF CHICAGO (NIH-69-2087)

Title: Definition of Sensitivity of Carcinogenesis Bioassay Systems

Contractor's Project Director: Dr. Stan Vesselinovitch

Project Officer (NCI): Dr. Jerry Rice

Objectives: To establish standard bioassay systems in mice for screening substances suspected of potential carcinogenic hazard to man, making best use of the age-dependent variation in sensitivity to chemical carcinogens exhibited by F_1 hybrid mice (first generation crosses of C3HeB x A/He and C57Bl/6 x C3HeB) and to provide definition of diagnostic criteria and of detailed biologic behavior of the neoplasms produced, especially those of the liver and lung.

Major Findings: The initial phase of these studies is essentially complete. This has involved administration of single intraperitoneal injections of benzo[*a*]pyrene or ethylnitrosourea, each at 2 dose levels, to newborn suckling and adult mice of both strains, and of repeated (4x) injections of diethylnitrosamine begun at the age of 6 week, 15 days or 1-24 hours. The results of these studies have shown a pronounced quantitative and qualitative age effect in the susceptibility of these animals to carcinogenesis. Animals treated with ethylnitrosourea at 1-24 hours of age have thus far exhibited more than 20 histological varieties of neoplasms, while those treated at later ages have exhibited only tumors of the kidney, lung, liver, and hematopoietic tissues. The tumor incidence and multiplicity for a given weight-adjusted dose of carcinogen is also greater in animals treated as newborns. A quantitative, but little or no qualitative difference, in carcinogenic response to the other compounds has also been demonstrated as a function of age at which exposure occurs. Although the results are not sufficiently complete to allow a choice at this moment between the two strains for routine use in NCI carcinogen screening programs, it is apparent that normal control animals of both strains at the age of 60 weeks have a very low incidence of spontaneous tumors. It follows that efforts should be made to develop regular testing protocols in which the much greater sensitivity of neonatal animals to chemical carcinogens would be utilized. Additional studies currently underway are designed to explore the sensitivity of these animals to known human carcinogens and to weak carcinogens of the pesticide type as a function of age and route of administration (including oral). Studies are also underway to determine the response of these mice to the transplacental administration of carcinogens.

Significance to Biomedical Research and the Program of the Institute: A major deficiency in carcinogen screening programs has been their lack of uniformity. It has been in the past quite impossible to estimate the relative carcinogenic potency of different compounds, even those tested in the same species, because of uncontrolled variables associated with testing protocols. These include the intrinsic differences in neoplastic response of different tissues in various strains of mice, the pronounced quantitative differences in response of fetuses, infants, and adults, and the marked effects of route of administration and dosage regimen. The purpose of this contract is to evaluate the significance of these variables within the context of an optimal screening program, and to develop improved standardized procedures which will be used for future carcinogen screening programs. Definition of biologic behavior and diagnostic criteria for the histologic identification of the neoplasms that can be induced in these animals will provide needed reference standards for the evaluation of endpoints in carcinogenesis tests.

Proposed Course: Studies currently underway in both strains of mice will be continued with particular reference to identification of any susceptibility to chronic neoplastic or non-neoplastic disease, which might preclude the satisfactory use of either strain of mouse in carcinogenesis screening programs. Future studies will be directed towards the utilization of the greater sensitivity of neonatal animals to develop model protocols for carcinogen screening programs. Studies will be initiated to specify clearly the biological nature of certain tumors and diagnostic criteria to be used in their identification. It will then be easier to evaluate the significance of certain of these lesions, particularly tumors of the liver, in assessing the possible hazard to man of substances capable of generating these lesions in the mouse.

Date Contract Initiated: June 25, 1969

Current Annual Level: $245,100

UNIVERSITY OF CINCINNATI (PH43-64-45)

Title: Determination of the Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals

Contractor's Project Director: Dr. Eula (Bingham) Mattheis

Project Officer (NCI): Dr. Richard Bates

Objectives: The purpose of this project is to test for skin carcinogenicity and co-carcinogenicity selected industrial chemicals to which there is significant human exposure.

Major Findings: A major portion of the effort of this contract is now devoted to expanding the important preliminary observation from this laboratory that optical brighteners enhance the carcinogenicity of ultraviolet light. These chemicals are widely distributed in the human environment since they are common ingredients in detergents, soap, and paper. In the initial studies, mice received skin applications of three optical brighteners and were exposed to an ultraviolet light with a maximum emission at 2,537 angstroms. Further studies, still underway, indicate that other optical brighteners besides the three initially tested have the same effect. There seems to be some difference in the extent of this effect among different brighteners, though final evaluation at this point must await completion of the experiments. Experiments are also underway to determine whether these chemicals enhance the carcinogenicity from other types of lights such as commercial sun lamp having a maximum emission at 3,100 angstroms and another light with a maximum emission at 3,500 angstroms.

Other experiments are to explore the co-carcinogenicity of aliphatic hydrocarbons observed in this laboratory when these were used as solvents for benzo[*a*]pyrene. The co-carcinogenicity of these hydrocarbons was dependent upon chain length. More recent observations suggest that these hydrocarbons may also be co-carcinogenic when used as solvents for pentachloronitrobenzene and that they are promoting agents for skin tumorigenesis after a single initiating dose of 7,12-dimethylbenz[*a*]anthracene but they do not enhance the carcinogenicity for the skin of β-propiolactone. In the case of pentachloronitrobenzene, the optimum chain length for co-carcinogenicity appears to be somewhat longer than it is for benzo[*a*]pyrene.

Significance to Biomedical Research and the Program of the Institute: The chemicals being tested in this contract are all ones to which humans are exposed. Thus, it is important to know whether they are carcinogenic. A most important function of this contract is to determine how combined exposure to various agents may affect their carcinogenicity. It has been demonstrated, for example, that chemicals which are not carcinogenic when tested by themselves may markedly enhance the carcinogenicity of other materials. This observation has very important implications for programs designed to restrict human exposure to carcinogens.

Proposed Course: The experiments outlined above will be brought to completion. They will be followed by other experiments on the co-carcinogenicity of industrial compounds.

Date Contract Initiated: September 11, 1963

Current Annual Level: $61,885

CITY OF HOPE MEDICAL CENTER (PH43-65-1036)

Title: Mycotoxins in Foodstuffs

Contractor's Project Directors: Dr. Eugene Roberts
Dr. Riojun Kinosita

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To investigate the carcinogenicity and toxicity of mycotoxins found in foods prepared by fermentation with microorganisms belonging to *Aspergillus* or *Penicillium* species and to analyze Ugandan foodstuffs for mycotoxins.

Major Findings: From samples of typical home-fermented Japanese foods, at least 40 strains of fungi have been isolated, identified and grown in different media. Some of these strains produced toxic materials which have not been identified. Culture filtrates of 13 strains, especially from *Aspergillus* *flavus*, produced some hepatomas on injection into male mice. In Ugandan foodstuffs 35 out of 140 samples contained aflatoxins at levels ranging from 6 to 820 ppb. From these foodstuffs over 400 strains of fungi were isolated. In 105 peanut samples from Uganda, aflatoxin levels ranging from 0 to 2,263 ppb were found in 13% of these samples.

Significance to Biomedical Research and the Program of the Institute: The presence of toxic fungi in home-fermented foodstuffs has been implicated as a possible explanation for the relatively higher incidence of gastric and liver cancer in Japan. Likewise, in African countries, such as Uganda, improper harvesting and storage conditions may lead to fungal contamination of foodstuffs perhaps a reason for higher liver cancer incidence. Proper identification of these fungi and the toxins produced by them will eventually lead to their control, thus eliminating a potential hazard to humans.

Proposed Course: Because of Dr. Kinosita's retirement for illness and disability, the contract will end June 30, 1971. However, all the toxic crystalline materials isolated from fungi and the corresponding fungal cultures have been transferred to Drs. Wogan and Büchi at the Massachusetts Institute of Technology for further investigation.

Date Contract Initiated: June 29, 1965

Current Annual Level: $85,167

COLUMBIA UNIVERSITY (PH43-67-731)

Title: Carcinogenicity of Fresh and Mildly Oxidized Vegetable and Animal Fats

Contractor's Project Director: Dr. Hans Kaunitz

Project Officer (NCI): Dr. Umberto Saffiotti

Objectives: This program was initiated in an attempt to determine the effects on general health, growth rate, tumor incidence and other disease conditions of the feeding to rats of purified diets containing specific fresh and mildly oxidized fats. The test materials include animal fats (butter, lard, chicken fat, beef fat) and vegetable fats (soybean oil, corn oil, cottonseed oil, and olive oil).

In addition, groups were fed soybean oil and lard after irradiation in an attempt to determine the safety of these materials in the diet.

Major Findings: The final results under this contract cannot be evaluated until the histopathology has been completed on all of the animals. It appears that observed differences in tumor incidences may be attributable to contaminants in the fats, rather than to the chemical nature of the different fats per se. Analytical data suggest the presence of nitrosamines as contaminants in some fats.

Significance to Biomedical Research and the Program of the Institute: Fats are major caloric constituents in man's diet and the safety of the various fats has been assumed to be established. There is need for more information on the minor constituents of fats in the diet and efforts are being made by the Carcinogenesis Area to explore the potential hazards of specific ingredients, in particular, fats of plant origin, and their oxidation products.

This contract fits into the overall program as a screening test on the carcinogenicity of dietary fat constituents.

Proposed Course: Completion of the program will include analytical determinations, final evaluation of pathology and a statistical analysis of results. Termination is expected by December 1971.

Date Contract Initiated: June 14, 1967

Current Annual Level: $57,555

CORDIS CORPORATION (NIH-69-2076)

Title: The Effects of Carcinogens on the Complement Non-Specific Immune System

Contractor's Project Director: Dr. Seymour Halbert

Project Officer (NCI): Dr. Tibor Borsos

Objectives: (1) To determine whether carcinogens are capable of inhibiting the action of any of the complement components *in vivo* and, if so, whether the process of inhibition could be used as a means of identification and characterization of carcinogens. (2) To determine whether the impairment of immune defenses that require the participation of complement is a factor in the etiology of cancer.

Major Findings: In short-term studies five carcinogens and their respective non-carcinogenic analogues were given intraperitoneally to groups of guinea pigs at doses in excess of levels reported to be carcinogenic. Blood samples were obtained before administration of the chemicals and after one day and seven days. In another experiment, blood samples were taken one hour, three hours, 24 hours and 48 hours after injection of the chemicals. There were no significant changes in complement component levels even in animals given the largest doses in spite of the apparent toxicity of the treatment.

The continued administration of the chemicals to guinea pigs in doses reported to induce carcinogenesis, have been studied thus far for two of the carcinogens and their corresponding non-carcinogens. The chemical was administered at a dose, and via a route previously shown by other workers to induce cancer. Blood samples were collected before administration of the chemical, after 2 weeks, 4 weeks and every 4th week thereafter.

The most striking finding in these groups of animals was the consistent and markedly reduced titers of C4 in animals given N-nitrosodiethylamine. The first group studied showed decrease in C4 levels as well as levels of certain other components except C5 and C7 which were unchanged. These animals progressively lost weight, did not survive longer than 8 weeks, showed hemorrhages in the lungs but showed no gross pathology in the liver.

Because of the possibility that the changes in complement component titers might be due non-specifically to the toxicity of the drug, a second group given half the original dose was started. In this group, the selective decrease of C4 was again clearly evident, and no other component changes were noted. In one animal that died during the period of observation, definite liver pathology has been noted. All other animals are healthy and gaining weight. This group is still under study and will be completed in another six months.

Animals receiving prolonged doses of the other carcinogen (N-hydroxy-2-acetylaminofluorene), as well as the non-carcinogenic analogues (N-nitrosodiphenyl amine and acetylaminofluorene), have continued to remain healthy and no significant changes in complement reactivity have been noted so far.

Significance to Biomedical Research and the Program of the Institute: This contract explores the effects of chemical carcinogens on the immune state of an experimental animal to determine the importance of the impairment of the immune response in the etiology of cancer. Ongoing in-house experiments indicate that tumors induced in guinea pigs by the water soluble carcinogen diethylnitrosamine are antigenic, and that these tumors can be rejected by the guinea pigs as a consequence of immunization with tumor antigens.

Proposed Course: It is expected that during the next fiscal year the contractor will study the *in vivo* effects on complement of chronic administration of carcinogens and their non-carcinogenic analogues. The contractor will also explore the *in vitro* effects of these chemicals in greater detail. Contractor will produce all the necessary reagents to carry out these tests; it should be appreciated that production of the reagents themselves represents a major undertaking.

Date Contract Initiated: June 21, 1969

Current Annual Level: $99,000

FRANKLIN INSTITUTE (NIH-71-2073)

Title: *Carcinogenesis Abstracts,* Volume IX

Contractor's Project Director: Dr. Miriam H. Fukami

Project Officer (NCI): Mr. Howard Rosenberg

Objectives: The publication of this abstract journal in the field of carcinogenesis provides an up-to-date abstract service for carcinogenesis researchers throughout the world. The format and content of the journal are designed to provide under one cover abstracts of published scientific work in the various disciplines which relate to cancer etiology.

Major Findings: Start-up time on *Carcinogenesis Abstracts,* Volume IX has been minimized. Rapid preparation of the first three issues has partially closed the inherited lateness gap at no sacrifice to quality of the publication.

Significance to Biomedical Research and the Program of the Institute: The Etiology Area of NCI, as it is now constituted, is made up of both intramural and contract research efforts in chemical and viral carcinogenesis and in biometry and epidemiology. The various scientific disciplines which are involved in or related to these investigations are many and varied. A great variety of researchers throughout the world contribute to the study of cancer etiology. The extreme difficulty of interrelating these diverse disciplines and the numerous factors that are, to some degree, implicated in carcinogenesis require that various mechanisms of communication between scientists of related disciplines to be identified and put into play. *Carcinogenesis Abstracts* has proven in the past to be a very useful and productive communication medium for both the researchers supported by NCI and others as well. A survey carried out on the use of and interest in this journal yielded a considerable positive response from working scientists. Investigators confirmed that the journal was valuable as an interdisciplinary alerting service as well as a vehicle through which to keep up with their own fields of endeavor.

Proposed Course: Present plans call for the continuation of this publication for an indefinite period of time. The Office of Management and Budget has approved publication and free distribution to qualified recipients through July 31, 1972. At that time the NCI will determine if application should be made for continuation of the journal. Periodic assessment of the abstracts for quality and relevance will continue to be made with the assistance of the Etiology staff. In addition comments and recommendations for improvement of the publication will be solicited from other sources. Recent efforts have focused on bringing the publication up to date, so that it may serve optimally as a current awareness tool. Currency with literature covered should be achieved early in Fiscal Year 1972.

Date Contract Initiated: January 7, 1971

Current Annual Level: $44,200

GENERAL FOODS CORPORATION (PH43-68-1005)

Title: Oxidation Products of Fats

Contractor's Project Director: Dr. R. J. Sims

Project Officer (NCI): Dr. Richard Yamamoto

Objectives: To isolate and identify intermediate and end products of fat oxidation and to study the chronic toxicity and carcinogenicity of some epoxides, specifically trivernolin (a triglyceride of *Vernonia* anthelmintia seed oil containing 70-75% 12,13-epoxy-9-octadecaroate) and alpha-cholesterol epoxide when administered as a component in food.

Major Findings: Among the products of oxidation of fat were the epoxides. Methods for identifying these epoxides, specifically fatty acid epoxide and alpha cholesterol epoxide were developed and utilized.

Trivernolin at 0, 1.0, 2.0, and 4.0% in the diet for 21 months followed by three months on control diet did not increase the incidence of tumor formation. Critical study of the pathology results is now being made.

Alpha cholesterol epoxide was fed at 0.5% and 1.0% level in the diet. The tissues have been processed and the data are being analyzed at the present time.

Significance to Biomedical Research and the Program of the Institute: Fats are oxidized under mild conditions such as cooking and heating. Some of the products of fat oxidations are toxic, as found to be the cause of chick edema disease, and produce compounds such as epoxide, lactones, and other oxidized compounds. Some epoxides and lactones have been found to be carcinogenic. The purpose of this program is to test some of these compounds for toxicity and carcinogenicity.

Proposed Course: This program should be completed this year since all of the animals have been killed and autopsied. Most of the histopathology is complete and awaiting tabulation and statistical analysis.

Date Contract Initiated: June 18, 1963

Current Annual Level: $49,347

GULF SOUTH RESEARCH INSTITUTE (NIH-70-2210)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. Harry Burchfield

Project Officer (NCI): Dr. Richard Bates

Objectives: The objectives of this project are to test a number of pesticides for carcinogenicity in mice and rats by administration in the diet.

Major Findings: The first set of 19 test and 2 control chemicals to be administered under this contract have been procured and analyzed for purity by using thin layer and gas chromatography, infra-red and mass spectroscopy and other procedures. These materials will be administered to the animals in their commercial form rather than being purified, since this is the form of human exposure. However, if some are found to be carcinogenic, the thorough analytical data which have been obtained will be highly useful for designing experiments to determine whether the carcinogenicity results from the pesticide itself or from its impurities. Subacute toxicity studies designed to determine the dosage level to be given in the long-term feeding experiments using C57Bl x C3H inbred mice and Osborne-Mendel random-bred rats are well underway and chronic experiments will begin in the near future.

Significance to Biomedical Research and the Program of the Institute: Many of the pesticides under study have widespread usage resulting in extensive human exposure to them. The chemicals selected for testing have either been tested inadequately for carcinogenicity in the past, or the results of such tests have been equivocal. These extensive experiments are designed to yield definitive data and help resolve these issues.

Proposed Course: Long-term feeding tests on the first group of compounds will begin soon and will continue for approximately two years. Chemical analysis, subacute toxicity and long-term feeding will be undertaken with a second group of compounds after the chronic experiments are underway with the first group. This program parallels Hazleton Laboratories (NIH-70-2219) in protocols but with different materials.

Date Contract Initiated: June 15, 1970

Current Annual Level: $212,600

HAZLETON LABORATORIES (NIH-70-2219)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. G. Carl Holsing

Project Officer (NCI): Dr. John Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity of environmental chemicals, with current emphasis on pesticides and related materials.

Major Findings: At this time 18 of the most widely used pesticides have been procured and have undergone chemical analysis. Materials will be administered in the commercial form rather than in a purified form since this is the form of human exposure. Evaluation of the subacute toxicity to determine the maximally tolerated dosages of the agents in C57Bl x C3H inbred mice and Osborne-Mendel randon-bred rats, to be used in the definitive studies, is presently being conducted.

Significance to Biomedical Research and the Program of the Institute: Because of extensive use, pesticides and related compounds are ubiquitous. Some of them have accumulated in man. Their involvement in the causation of human disease is virtually unknown. The purpose of this program is to gather fundamental data so that adverse effects in man can be predicted, and hopefully prevented.

Proposed Course: This program is now in its initial stages and parallels Gulf South Research Institute (NIH-70-2210) in protocols but with different materials. Upon completion of the preliminary toxicology with all agents, the main groups of animals will be phased in, beginning probably late in 1971. Present plans call for an 18-month feeding and an additional 6-month holding period. The overall program is expected to continue until approximately 1974.

Date Contract Initiated: June 8, 1970

Current Annual Level: $222,600

IIT RESEARCH INSTITUTE (NIH-70-2245)

Title: Production and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis

Contractor's Project Director: Mr. Reg Davies

Project Officer (NCI): Dr. John Cooper

Objectives: The objectives of this program are to provide services to the National Cancer Institute's Special Lung Cancer Program in several specific areas of particle technology, to prepare and characterize the following materials: (1) powder fractions, differing in size, composition and surface area; (2) powder fractions coated with selected polynuclear hydrocarbon carcinogens; (3) dispersions of coated particles in selected vehicles; and (4) stable emulsions of selected polynuclear hydrocarbon carcinogens in selected vehicles.

Characterization of the samples includes the following determinations: (a) purity of the sample as determined by emission spectroscopy; (b) the actual particle size distribution of the sample; (c) information on the conformation of particles in the sample as determined using the scanning electron microscope; (d) a surface area measurement performed on the sample by inert gas adsorption.

Major Findings: (1) *Particle Preparation* - Forty gram quantities (2 batches of 20 gram each) of carbon, ferric oxide, alumina, nickel oxide, and cobalt oxide have been prepared in four size ranges with 90% of the particles falling within the specified limits of 0.5-1.0 microns, 2-5 microns, 5-10 microns and 15-30 microns. The cascade sedimentation method was used. At the present time only the second batch of nickel oxide remains to be prepared, and fractions of all other materials are in current use in respiratory studies. No significant differences were found between the samples analyzed from the first and second sets of 20 gram batches.

(2) *Particle Coating* - Particles of carbon, hematite and alumina have been coated with benzo[*a*]pyrene (BP) and have been supplied to the National Cancer

Institute, Massachusetts Institute of Technology, Ohio State University, and IIT Research Institute's Life Sciences Division. Over 60 coating preparations have been made to date, and on each sample photomicrograph characterization for free BP and the degree of agglomeration has been made.

Free BP in the form of needles is still found to exist in some preparations and no conclusive explanation can be given for the phenomenon at this time.

(3) *Dispersion of Particles in Liquids* - Due to the sedimentation instability of BP-coated particles in gelatin-saline vehicles during transport, all coated particles have been supplied as dry powders. In all cases, samples of control dust have been submitted at the same time.

In all, 400 preparations of this kind have been prepared, and these samples are scheduled to continue for the remainder of the current support period.

(4) *Preparation of Emulsions* - In some experimental programs, BP was administered as a ball-milled preparation (emulsion) in the presence of gelatin and saline. Various BP loadings were prepared as requested, approximately 40 preparations have been made. All of these preparations have been characterized with respect to the BP content and particle size distribution.

Significance to Biomedical Research and the Program of the Institute: This contract is intended to produce the particulates required for the execution of other contracts in the Carcinogenesis Program. In addition, this effort will provide investigative and production support for such contracts, in the areas of fine particle technology and stable emulsion preparation and characterization.

Proposed Course: It is intended that this contract be continued as a production and service resource for related contract activities in this area. It is anticipated that the number of preparations made for the various respiratory carcinogenesis programs will increase from an estimated 1,000 in Fiscal Year 1970-1971 to 1,500 in Fiscal Year 1971-1972.

The workscope for the coming support period will include: (1) The preparation and characterization of the 20 powder fractions in excess of 40 grams will continue as it is anticipated that the demands for these materials will increase. (2) The preparation of coated fractions of particles will continue. Here, it is expected that other carcinogens will be introduced, e.g. pyrene, dibenzcarbazole. (3) The preparation of hand ground mixtures of particles and carcinogens will be more prominent during the next fiscal year as already interest in these materials is growing. (4) The preparation of suspensions of particles in various vehicles will proceed and will increase. In particular, long-term studies using cobalt and nickel oxides are planned. (5) Research studies into better mechanisms of powder coating will be initiated, as this problem has been acute during recent studies. And (6) A research study into the degradation rate of carcinogen in all preparations will be initiated. It is essential that the extent of degradation and actual concentration of BP be measured after preparation and normal storage.

Date Contract Initiated: June 10, 1970

Current Annual Level: $51,283

IIT RESEARCH INSTITUTE (NIH-69-2148 and Supplement)

Title: Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay Using Intratracheal Instillation Techniques in Syrian Golden Hamsters

Contractor's Project Director: Dr. Mary Henry

Project Officer (NCI): Dr. David Kaufman

Objectives: To determine the effect of intratracheal instillation of particulates of varied chemical and physical properties and of various types of solvents on the induction of epidermoid carcinoma of the lung by chemical carcinogens in the hamster.

Major Findings: Chemically and physically defined particulates have been produced by another contract at this institution (NIH-70-2245). Long-term carcinogenesis studies are now in progress to determine the effect of these particles of different size and composition on the induction of lung tumors by diethylnitrosamine. Ancillary studies on vehicles for carcinogen administration have shown that benzo[*a*]pyrene suspended in a gelatin-saline solution can produce lung tumors; similar studies by others had previously failed to demonstrate this. In addition, short-term studies in progress are studying the influence of various particles on the rate of disappearance of benzo[*a*]pyrene following intratracheal instillation.

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that bronchogenic epidermoid carcinomas could be induced in hamsters by the intratracheal administration of benzo[*a*]pyrene with iron oxide dust and that lung tumors can be induced by subcutaneous injection of diethylnitrosamine followed by intratracheal instillation of dusts. Further elaboration of these observations is needed to determine the role of atmospheric dusts or cigarette smoke particulates on the development of bronchogenic carcinoma in man.

Proposed Course: The project will continue with long-term carcinogenesis studies designed to determine the essential characteristics of particles as they affect the induction of epidermoid carcinoma of the lung. Supplemental efforts will be directed toward determining the effects of particulates, carcinogens, vehicles, irritants, and vitamin A, as they relate to changes in morphology of respiratory epithelial surfaces as viewed by scanning electron microscopy.

Date Contract Initiated: June 27, 1969

Current Annual Level: $148,705

LIFE SCIENCES, INC. (NIH-69-2056)

Title: Viral and Chemical Co-Carcinogenesis

Contractor's Project Director: Dr. Richard Donovick

Project Officers (NCI): Dr. Jerry Rice
Dr. Padman Sarma

Objectives: This contract was initiated for the purpose of exploring approaches to the study of viral-chemical co-carcinogenesis in avian tumor virus systems. These viruses, selected for study because of their rapidity of action and high state of development as experimental models, include the standard strain of Rous sarcoma virus (RSV) which is defective in most strains of chickens and requires an accompanying helper virus: BS-RSV (RAV 1); the Schmidt-Ruppin strain of RSV which is not defective: SR-RSV; and finally, the helper-free strain of RSV which is infective for Japanese quail in the absence of helper: RSV(O). Avian hosts being tested for suitability in these *in vivo* studies were: conventional chickens from commercial sources; leukosis-free SPF chickens; and leukosis-free SPF Japanese quail *(Coturnix)*. Chemical carcinogens used were selected by NCI project officers and supplied by NCI; they included dimethylnitrosamine, diethylnitrosamine, propane sultone, ethylnitrosourea, 1-nitro-3-nitroso-3-methyl guanidine and 7,12-dimethylbenz[*a*]anthracene.

Major Findings: Short-term studies were carried out in both chickens and quail with various tumor virus preparations and each of the above carcinogens. A variety of doses of both viral and chemical agents were used, and a series of protocols were employed to administer the chemicals at various times relative to infection with virus. End point of these short-term studies was the appearance of virus-induced tumor at the site of virus inoculation. No consistent shortening of the latent period for viral infection, that is, for the development of virus-induced tumors, was found to result from pretreatment of the host with any of the above agents. Some regimens provided a very slight shortening of latent period, and could be said to have enhanced the viral infection; in other cases, the latent period was significantly prolonged as a result of chemical treatment. The absence of any reproducible correlation between chemical carcinogen treatment and shortening of latent period for viral oncogenesis discourages the concept that such studies could provide a useful short-term screen for potential chemical carcinogens.

It had also been hoped that long-term carcinogenesis studies might be undertaken in SPF quail latently infected with a subthreshold dose of avian tumor virus, with the aim of determining whether subsequent chemical carcinogen administration might result in activation of the latent oncogenic virus infection. Such studies would be expected to give rise to a different spectrum of tumors, with different latent periods, than would have been obtained in controls given the chemical carcinogens alone. Unfortunately, it was not possible for NCI and the contractor to come to terms concerning these protocols.

Significance to Biomedical Research and the Program of the Institute: The failure of the short-term oncogenic viruses to be reproducibly and consistently affected with respect to virulence as a result of chemical carcinogen pretreatment of the host suggests that this approach to the rapid screening of potential human carcinogens will not be fruitful.

Proposed Course: Termination

Date Contract Initiated: April 17, 1969

Current Annual Level: $142,100

MARQUETTE SCHOOL OF MEDICINE (NIH-69-2082)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor's Project Director: Dr. Hollis Boren

Project Officer (NCI): Dr. Curtis Harris

Objectives: The objective of this project is to determine the proliferative cellular response of various segments of the respiratory tract following exposure to respiratory carcinogens and particulates, using primarily the hamster intratracheal instillation model which is studied in other respects in several other contracts. The labelling response is calculated by means of autoradiographic measurement of tritiated thymidine uptake in alveoli and in bronchi of different diameter. These measurements are correlated with the histopathological response to carcinogens and particulates.

Major Findings: Work accomplished in the first year demonstrated specific time peaks for the cellular response to intratracheal instillation of benzo[*a*]pyrene, ferric oxide, or their mixture in single or repeated doses and with different sequence.

In the second year, a number of carcinogens, particulates and solvents were compared. Dibenz[*a,h*]anthracene and methylcholanthrene caused a smaller increase in DNA synthesis than benzo[*a*]pyrene. The carrier dust, iron oxide, produced a significant increase in DNA synthesis, whereas titanium dioxide caused no response. Other contracts are evaluating the co-carcinogenicity of carrier particulates including titanium dioxide. The intratracheal instillation of flurocarbon solvent, FX80, produced a marked increase in DNA synthesis. An increased incorporation of tritiated uridine preceded the peak of DNA synthesis by 12 hours.

Significance to Biomedical Research and the Program of the Institute: This contract is an integral part of an interlocking group of contracts designed to define the role of carcinogens and the physico-chemical factors (e.g., particulates) required for respiratory carcinogenesis. The other studies, including long-term tests have been designed so that they are closely related to the present one with maximum interaction but no overlap of activity, in an effort to define the key role of vehicles and particulates in the process of respiratory carcinogenesis.

Proposed Course: Continuation of these studies will assess the importance of particle size of ferric oxide on its induction of DNA synthesis. The effect of hypervitaminosis A on tumor histogenesis and DNA synthesis will also be investigated. Collaborative studies with the Lung Cancer Unit, NCI, will investigate the effect of selected carcinogens on RNA maturation and transport in the tracheal epithelium.

Date Contract Initiated: June 3, 1969

Current Annual Level: $67,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (PH43-62-468)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald Wogan
Dr. George Büchi

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To investigate the biochemical and carcinogenic effects of aflatoxins, their analogs and their metabolites, to determine the metabolic fate of aflatoxin in various species, to synthesize the aflatoxin metabolites and to determine the biosynthetic pathway of aflatoxin.

Major Findings: Improved syntheses of certain of the aflatoxin metabolites, such as M_1 (hydroxy B_1) have enabled the MIT group to study the toxicity of this compound and its biochemical action. Monkeys dosed with aflatoxin B_1, excreted an O-dimethyl derivative. Levels of this material in the urine of humans eating moldy foods might be used as a screen for indication of the aflatoxin B_1 level of the foods. The structures of certain compounds associated with a very toxic strain of *A. glaucus* have been determined.

Significance to Biomedical Research and the Program of the Institute: Adulteration or alteration of foodstuffs by microbiological agents may be of considerable importance as a cause of liver cancer in certain areas of Africa or Asia. This project is related to other contracts on natural products supported by NCI.

Proposed Course: The experiments on various phases of mycotoxin research will continue. Studies on the metabolism in various species may help delineate the reasons for the varying responses to aflatoxin. Determination of the biosynthetic pathway of aflatoxin may give leads on blocking the growth of the toxin-producing species.

Date Contract Initiated: June 25, 1962

Current Annual Level: $140,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-70-2180)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractor's Project Directors: Dr. Gerald Wogan
Dr. Steven Tannenbaum

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: Reports of the apparent presence of nitrosamines, multipotential carcinogens, in several foods and beverages, raised the question of the sources of these contaminants and whether these reports may have been based on artifacts. The contractor is therefore developing methods for determination of both volatile and nonvolatile N-nitroso compounds in foods and studying the conditions favorable to their formation in foods or model food systems.

Major Findings: Methods have been devised to isolate relatively volatile nitrosamines from food samples. An automatic analytical method for nitrosamines is operating. Syntheses of certain model nitrosamines which might occur in foods have been carried out. In addition kinetic studies of the conditions under which nitrosamines are formed or decompose have been made.

Significance to Biomedical Research and the Program of the Institute: Since N-nitrosamines have proved to be multipotential carcinogens in all animal species tested they may represent significant hazards to man as environmental contaminants contributing to the "spontaneous" cancer incidence. This project will aid in determining the total extent to which nitrosamines may be present in the environment and the conditions favoring their formation during processing and storage. Thus measures to prevent this formation could be instituted.

Proposed Course: The project will continue along the same lines, attempting to refine analytical techniques for nitrosamines and determining conditions favoring their formation.

Date Contract Initiated: June 11, 1970

Current Annual Level: $45,700

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-69-2083)

Title: Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract

Contractor's Project Director: Dr. George Wolf

Project Officer (NCI): Dr. Michael Sporn

Objectives: It has been observed that excess vitamin A inhibits not only squamous metaplasia but also the development of squamous cell carcinomas from the bronchial mucosa in benzpyrene-treated hamsters (Saffiotti *et al.*, 1967). The extent of this inhibitory action, the biochemical pathways on which it depends, and the relationship of differentiation to carcinogenesis can be investigated by using a rapidly effective form of vitamin A (retinoic acid) and transplantable tumors as source of tissue.

The aims of the project are as follows: (1) to determine the effective conditions by which vitamin A inhibits the development of squamous-cell carcinoma from the bronchial mucosa; (2) to determine the effect of vitamin A levels on the control of mucous-squamous differentiation and growth patterns in transplantable bronchogenic carcinomas.

Major Findings: Two main long-term studies in hamsters are in progress: (a) to investigate the effect of excess retinol and retinoic acid on benzo[*a*]pyrene-induced transplanted anaplastic tumors of the lung. The end points of excess retinol and retinoic acid are being defined both histologically and biochemically; (b) to study the effects of excess retinol and retinoic acid on benzo[*a*]pyrene-induced squamous cell carcinoma of trachea and bronchi.

A major achievement in the study of the control of mucous-squamous cell differentiation by vitamin A has been the discovery that retinol is necessary for the biosynthesis of a specific glycopeptide containing fucose, galastose, glucosamine, sialic acid, uronic acid and 15% protein. This glycopeptide was shown to be located only in goblet cells of the intestinal mucosa by the indirect immunofluorescence method. This bears special significance to the problem of cell differentiation since it was found that both in intestinal and tracheal epithelium the number of goblet cells drastically decreases in vitamin A deficiency. Hence the immunofluorescence assay can be used as a marker for mucus cell formation. Moreover, it was found that the synthesis of the same glycopeptide was severely decreased in vitamin A deficient lung tissue. Fucose-glycopeptide from the respiratory tract was prepared by DEAE-Sephadex, Sepharose 4B and Sepharose 2B chromatographies, and its purity was tested by polyacrylamide gel electrophoresis. Studies on transplanted anaplastic carcinomas of the bronchus (ALY-241) have shown that retinoic acid given orally to the host can drastically change the relative distribution of C^{14} glucosamine labeled glycopeptides eluted from DEAE-Sephadex A50.

Significance to Biomedical Research and the Program of the Institute: The project represents an essential component of the Lung Cancer Program currently developed by the Carcinogenesis Area following recommendations of the Lung

Cancer Task Force. The methods used for carcinogenic treatments are closely related to those used for the other projects in the program on the processes of respiratory carcinogenesis, the role of vehicles and particles, the measurement of cellular responses, and long-term carcinogenesis assays on various inhalants and respiratory carcinogens. The findings expected from this project are highly relevant to the interpretation of the response of the respiratory tract to carcinogens and controlling factors, and to the development of assay studies for tobacco constituents. The potential value of a method for the effective inhibition of respiratory squamous carcinogenesis needs no further elaboration. In the study of the mode of action of vitamin A in the control of mucous-squamous cell differentiation, a biochemical marker for goblet cell differentiation, the fucose-containing glycopeptide, has been found. The biosynthesis of this specific macromolecule depends on vitamin A and its presence in tissues may easily be assayed by immunofluorescence.

Proposed Course: To continue the long-term animal studies already begun, so that complete evaluation of the gross and microscopic pathology can be made. To continue with biochemical studies on the role of vitamin A in controlling normal differentiation of respiratory epithelium.

Date Contract Initiated: June 18, 1969

Current Annual Level: $74,000

MELOY LABORATORIES (NIH-70-2201)

Title: Definition of Standard Experimental Conditions for a Reproducible *In Vitro* Carcinogenesis Bioassay

Contractor's Project Director: Dr. John Verna

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: The goal of this project is to establish fast, reproducible, standard *in vitro* methodology for determining potential carcinogens in man's environment.

Major Findings: A known method for rapidly detecting carcinogenesis is the observation of the altered colonial morphology of transformed cells. Therefore, priority has been given to the determination of the optimum experimental conditions for growth and recognition of normal and transformed colonies of the selected cell type in order to provide the fundamental criteria for distinguishing toxic and transforming effects of chemicals.

After selecting potentially susceptible cells and chemical agents, testing different medium compositions, and comparing several experimental procedures, a set of conditions for *in vitro* chemical transformation has been established.

Transformation has been observed with a number of polycyclic hydrocarbon carcinogens using NIH/3T3 cell lines. The tumor producing ability of DMBA transformed NIH/3T3 cell line (NT72) together with positive and negative controls was demonstrated. Transformed NIH/3T3 showed positive tumorigenicity in mice, both irradiated and non-irradiated. X-irradiation seemed not to influence tumorigenicity, except for the tendency to develop more uniform size tumors. Some of the animals will be observed to determine whether or not they recover from once developed tumors (regression) or succumb to tumors (tumor death). Other animals with tumors will provide the materials for histology and for *in vitro* recultivation in order to make comparison between the original and recovered cells.

Significance to Biomedical Research and the Program of the Institute: The total program aims at demonstrating the usefulness of *in vitro* assays for chemical carcinogens. This research has direct relevance to the etiology of cancer. The development of a series of *in vitro* assays should enable cancer investigators to rapidly determine the carcinogenicity of a wide variety of chemicals as well as to carry out fundamental studies related to the process of carcinogenesis. In this fashion an entirely new approach will become available for the study of chemical carcinogenesis.

Proposed Course: During the course of the first renewal, it is proposed that additional cell lines and carcinogens be employed to establish the range of carcinogen detectable by *in vitro* bioassay. Carcinogens previously demonstrated to be carcinogenic *in vitro* and *in vivo* by other investigators will be examined in those assay systems. Compounds considered to be non-carcinogenic will also be examined. As originally proposed the role of chemicals, oncogenic, and non-oncogenic viruses as enhancers of chemical carcinogenesis will be studied. The degree of confidence in the assay systems will be determined by using double blind techniques.

Date Contract Initiated: June 26, 1970

Current Annual Level: $132,447

MICROBIOLOGICAL ASSOCIATES (NIH-70-2199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Relating Activities

Contractor's Project Director: Dr. Russell Madison

Project Officer (NCI): Dr. Jerry Rice

Objectives: This contract provides for laboratory support services for the Carcinogenesis Area, NCI, in the field of experimental pathology. The project is intended to provide the following services under the direct supervision of the NCI staff: (1) perform bioassay studies according to protocols developed especially by NCI, including controlled feeding, parenteral administration, skin painting, intragastric intubation, intra-tracheal instillation and other techniques as required, using mice, hamsters and rats; (2) perform animal treatments and maintain precise observation records as prescribed by individual NCI staff members who have initiated the protocols for different types of carcinogenesis studies; (3) conduct gross and histopathological examinations and evaluations of experimental animals according to NCI specifications, and to provide a measure of histological support for NCI in-house activities; (4) provide photographic services as required by the project and other NCI needs.

Major Findings: During this first year of the contract, 20 separate protocols were initiated in rats, mice, hamsters and rabbits, 4 of which were terminated within the first year. These protocols were initiated by 7 different investigators within the Carcinogenesis Area, and have involved feeding studies, intratracheal instillation methods, and parenteral injections in approximately 900 rats, 1600 mice, and 1100 hamsters. The major areas of activity include the following: (1) intratracheal instillations of carcinogens and various forms of dust in hamsters, in support of studies carried out by the Lung Cancer Unit, OASDC; (2) oral administration of carcinogens and related non-carcinogenic compounds in normal and protein deficient diets, in support of studies carried out by the Carcinogen Screening Section, Experimental Pathology Branch, and the Nucleic Acids Section, Chemistry Branch; (3) parenteral administration of compounds, both carcinogenic and non-carcinogenic, for the exploration of various aspects of chemical carcinogenesis by the Bioassay Section and the Histopathology Unit, Experimental Pathology Branch.

Significance to Biomedical Research and the Program of the Institute: This contract provides an essential facility for the initiation and completion of long-term experiments involving large numbers of experimental animals. As an extension of NCI in-house research, the facility provides a means for rapid testing of new ideas in experimental pathology related to chemical carcinogenesis.

Proposed Course: This contract should be renewed and expanded slightly during the coming year. There is need for an additional professional to act as a direct assistant to Dr. Madison, and there is need for expansion of the laboratory facilities which will be used primarily to provide samples of pulmonary tissues previously subjected to carcinogenic regimens to NCI staff members for biochemical studies.

Date Contract Initiated: June 4, 1970

Current Annual Level: $246,262

UNIVERSITY OF MINNESOTA (NIH-70-2058)

Title: A Study of the Levels of Polycyclic Hydrocarbons in An Urban Atmosphere

Contractor's Project Director: Mr. George Carson

Project Officer (NCI): Mr. Raymond Newell

Objectives: To study, over a range of particle size fractions, the concentration of polycyclic hydrocarbons in an urban atmosphere.

Major Findings: A high flow rate, multistage air sampler has been developed. A prototype has been fabricated and is currently being used in environmental sampling.

Significance to Biomedical Research and the Program of the Institute: This program will provide information as to the concentration of polycyclic hydrocarbons that may be contained in the respirable fraction of urban air components. This method of sampling might be used advantageously for a number of airborne carcinogenic materials.

Proposed Course: This contract will be completed in 1971.

Date Contract Initiated: December 31, 1969

Current Annual Level: $5,000

UNIVERSITY OF NEBRASKA (PH43-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio Gori

Objectives: The purpose of this contract is two-fold in nature: the bioassay of agents suspected of being carcinogenic, and fundamental research on mechanisms of carcinogenesis.

(1) *Bioassay* - Compounds are selected for carcinogenic screening on the basis of several criteria, including (a) suspicion as a result of epidemiological studies uncovering suspected high risk to cancer in defined environmental exposures; (b) chemical properties and structures or physical properties suggesting a high index of suspicion for potential carcinogenic potency, and (c) potential or actual universal distribution or use of agents suggesting the need for carcinogenic bioassay. The techniques of bioassay include administration by various routes. Selection of various modes of administration is determined by properties of the materials, comparative response in various species, determination of dose response parameters, and the desire for maximum extrapolation to man. Protocols for these experiments take into account the current state of knowledge in the field as well as the properties of the agents being tested. Experimental design also utilizes the skills of the chemistry group and provides data analysis systems. A large number of compounds are under bioassay, using several dose levels and multiple species.

(2) *Research on the Mechanism of Carcinogenesis* - Objectives include fundamental research at the chemical and host levels for the purpose of elucidating biological mechanisms involved in the carcinogenic process and to identify the factors controlling or modifying the natural history of

induced neoplasms. Methods employed include the determinations of anatomic and metabolic fates of environmental carcinogens to determine sites of elective localization and paths of detoxification and excretion. Identification of the active form of the carcinogen, characterization of hormonal or genetic host factors critical to the carcinogenic responses, and comparison of response in different species are integral parts of this program. Molecular aspects of carcinogenesis are actively investigated.

Major Findings: (1) *Bioassays* - The status of the bioassay efforts may be summarized as follows: (a) Six additional drugs tested have proved negative for carcinogenicity; one is under suspicion and results are being analyzed. (b) The testing of 11 components of cosmetics has been initiated on the skin of mice and rabbits. (c) Three groups of food additives or contaminants are under test, including artificial sweeteners in hamsters, aflatoxins in rats, and nitrites in combination with various amines in mice and rats. In mice piperazine, morpholine, and methylaniline in combination with sodium nitrite significantly enhanced the development of lung adenomas. The results with nitrites and amines in rats are expected in mid-1971. The tests with artificial sweeteners are at an early stage and the aflatoxin studies are finishing. (d) Lung bioassays of eight aromatic hydrocarbons (some of which are components of tobacco tar), six metallic dusts in combination with a carcinogen, and seven carcinogenic compounds in various combinations are underway in hamsters. Potent carcinogens for the respiratory tract were found to be 7-H-dibenz[*c,g*]carbazole and dibenzo[*a,i*] pyrene were found to be potent carcinogens for the respiratory tract. (e) Comprehensive tests of seven hydrazine analogs for carcinogenicity in mice, hamsters, and guinea pigs have yielded a variety of tumor types indicating a structure-dependent selective organ response.

(2) *Research on the Mechanism of Carcinogenesis* - Selective examples of research projects include the following: (a) The mechanism of lung carcinogenesis continues to be studied, with emphasis on the definition of lung cancer models in animals and the importance of biological background in lung tumor induction by studies of causes of different spontaneous tumor levels in mice. Milk was found to be an effective carrier of two nitrosamine carcinogens in hamsters: oliethyl- and dibutynitrosamines, given to lactating mothers, induced respiratory tract tumors in the sucklings. (b) The chemical and biological action of nitroso compounds, a class of potent carcinogens, is being investigated. Studies include conditions of formation of nitrosamines from nitrite and secondary or tertiary amines, analysis of the occurrence of nitrosamines and their precursors in the environment, metabolism of nitroso compounds in several animal species and tumor induction in specific organs by compounds of different structures. (c) Development of test methods and models for studies in chemical carcinogenesis continued. Studies of differences in host response due, for instance, to bacterial flora or the age and sex of the animal are undertaken for comparison with the situation in man. Intravenous injection of 7,12-dimethylbenz[*a*]anthracene in hamsters was found to induce a high incidence of ovarian tumors.

Significance to Biomedical Research and the Program of the Institute: This contract has produced a high output of important major research results in chemical carcinogenesis over the years. In addition, it has provided considerable training in a field not very popular with the majority of investigators, partly because of the long-term nature of many of the experiments, and partly due to the frequency of negative results. The contract provides a major national resource in the field of environmental carcinogenesis. Its program is closely related to the whole NCI program in this field.

Proposed Course: The project will continue with the following three main objectives: (1) Bioassay activities on compounds of environmental significance. Approximately 75% of contract resources will be devoted to testing. Selection of compounds, experimental design, evaluation end points and criteria will be determined in strict collaboration with NCI. (2) Research in carcinogenesis which will focus on improved design and interpretation of bioassay, and on the understanding of the underlaying biological mechanisms. (3) Training of graduate students and postdoctoral scientists in the field of carcinogenesis, with particular emphasis in bioassay design and interpretation.

Procedures for clearer and more frequent communications with NCI have been established. A detailed cost accounting is being instituted. These measures will insure a closer intergration of the project with NCI activities and objectives.

Date Contract Initiated: March 19, 1968 (formerly Chicago Medical School--December 1, 1964)

Current Annual Level: $1,030,000

UNIVERSITY OF NEBRASKA (NIH-70-2203)

Title: Development of An *In Vitro* Test for Chemical Carcinogenesis

Contractor's Project Director: Dr. Melvin Greenblatt
Dr. Gioampiero diMayorca

Project Officer (NCI): Dr. Gio Gori

Objectives: The project will endeavor to develop experimental conditions for a sensitive and reproducible *in vitro* bioassay for the determination of the carcinogenic potential of chemical compounds. The approach will include the following: (1) Diploid cells will be utilized to avoid the possibility of dealing with cancerous cells prior to treatment. (2) Cells exposed to the chemicals tested will be cloned to reach a precise quantification of transformed individual cells. (3) Some chemicals are known to undergo metabolization *in vivo*, leading to production of active carcinogens. Presumably these chemicals would be inactive in an *in vitro* bioassay. To overcome this difficulty, the detector diploid cells will be exposed, in implanted semipermeable diffusion chambers, to the body fluids of animals treated with the chemicals under test. After such exposure the diploid cells will be cloned for quantification of transformation events. (4) Using a number of compounds known to be positive or negative for carcinogenicity, a rigorous correlation will be established between the ability of transforming cells *in vitro*, and the corresponding carcinogenic activity in animals. (5) Attempts will be made to standardize *in vitro* bioassay conditions such as to be producible under routine laboratory conditions.

Major Findings: Major accomplishments have been the standardization of *in vivo* and *in vitro* techniques, the development of a superior diffusion chamber, and the selection of the initial compounds to be evaluated. Chemicals selected for the initial pilot study are three members of the nitroso class of chemicals: (1) nitrosomethylurea - a powerful mutagen and transforming agent which produces local tumors at the site of injection in impact animals; (2) dimethylnitrosamine - a systemic carcinogen which apparently requires metabolic activation for its action; and (3) diphenylamine - a nitrosamine exhibiting no transforming or carcinogenic action. Preliminary experience with these agents, which have concentrated on establishing dose response relationships using plating efficiency as an index of toxic effects, have established the sensitivity of BHK21 and RCL3 cells to nitrosomethylurea in *in vitro* and *in vivo* studies. RCL3 cells appear to be more sensitive *in vivo* to the toxic action of NMU than RBHK21 cells when both cell lines are exposed to equimolar doses of a test compound delivered to the peritoneal cavity. Dimethylnitrosamine, at the relatively high concentration used did not affect the plating efficiency of exposed cells either *in vivo* or *in vitro*. Delayed toxicity for MNU complicated quantitation of transformation of events expressed per surviving cell but some clones in both *in vivo* and *in vitro* experiments appear transformed by this agent. These experiments have just recently been started and the results are still too preliminary to arrive at any firm conclusion.

Significance to Biomedical Research and the Program of the Institute: This project will integrate in the activities of the Chemical Carcinogenesis Area. It will contribute to the much needed development of rapid and inexpensive bioassay methods for testing or screening of chemicals. It will also contribute to the program needs for biological models for carcinogenesis studies at the cellular level.

Proposed Course: To continue as outlined above.

Date Contract Initiated: June 26, 1970

Current Annual Level: $96,425

NEW YORK UNIVERSITY (PH43-66-962)

Title: Pulmonary Carcinogenesis

Contractor's Project Director: Dr. Marvin Kuschner

Project Officers (NCI): Dr. Umberto Saffiotti
Mr. Raymond Newell

Objectives: The conditions required for the induction of bronchogenic carcinomas and other pulmonary tumors are tested in a series of experiments using administration of polynuclear hydrocarbons (methylcholanthrene and benzo[*a*]pyrene) to rats and hamsters by the following techniques: (1) intrabronchial pellet implantation; (2) intratracheal instillation of fine suspensions; and (3) inhalation exposure to finely dispersed particles in specially designed chambers.

Using these biological models, additional problems studied include: (a) determination of the synergistic effects of sulfur dioxide and nitrogen dioxide inhalation with benzpyrene carcinogenesis; (b) Halo-ethers - Inhalation tests with chloromethyl ether and bis(chloromethyl)ether will be completed; (c) Chromates - Intrabronchial pellets filled with calcium chromate have induced bronchogenic squamous cell carcinoma at the site of implantation. Inhalation tests are now underway; (d) Fiberglass - Intratracheal instillation tests of fiberglass-reinforced plastics have so far proven negative for tumorigenic effects. A new apparatus has been developed for the production of atmospheric dispersions of fiberglass-plastic particles and pilot inhalation tests have been conducted; (e) Polyurethane - Similar exposures are used for polyurethane plastics; (f) All these tests involve supporting studies of particle size, dispersion techniques, monitoring of inhalation exposures and analytical techniques; (g) In addition to pathological study of the test animals, tissue cultures of pulmonary macrophages (up to seven days) are used to test toxicity and phagocytic activity; (h) a new series of tests has been set up using intrabronchial pellet implantation of tobacco smoke condensate in rats, with or without addition of benzo[*a*]pyrene.

Major Findings: The techniques for administration of polynuclear hydrocarbons through the respiratory tract have been further developed and analyzed. The role of sulfur dioxide in enhancing respiratory carcinogenesis when inhaled together with benzpyrene has been indicated by preliminary findings now being controlled and repeated.

Similar tests with nitrogen dioxide are underway. In the long debated question of the carcinogenic potential of chromates, the demonstration of topical induction of squamous carcinoma by intrabronchial implantation has thrown new light on the problem, focusing on effective dose levels: inhalation tests are now in progress.

A series of implant, intubation, and inhalation studies relating to the biological effect of polyester-fiberglass and polyurethane plastics has been concluded. Inhalation of polyurethane dust for only 30 days in rats led to subsequent development of carcinomas of the lung. This striking finding is being followed up. Long-term inhalation studies of halo ethers are underway after demonstration of marked carcinogenic effects of bis(chloromethyl)ether in the respiratory tract of rats. Also in progress is a study of tobacco tar exposure by intratracheal pellet exposure.

Significance to Biomedical Research and the Program of the Institute: This contract represents a component of a broad effort to develop bioassay techniques for studies on lung cancer; it is also important for its study of potential hazards of materials that are in use industrially and may be found in the environment.

Proposed Course: The present projects of this contract are under terminal support because of the Principal Investigator's change of institution. It is proposed to continue support until all experimental work is completed and adequately reported.

Date Contract Initiated: June 29, 1966

Current Annual Level: $278,221

NEW YORK UNIVERSITY (PH43-64-938)

Title: Tumor-Promoting Principles in Tobacco and Tobacco Smoke

Contractor's Project Director: Dr. Benjamin Van Duuren

Project Officer (NCI): Dr. Harry Gelboin

Objectives: The major goals of this study are the isolation and identification of tumor-promoting agents derived from tobacco and tobacco smoke condensates and the determination of their contribution to the health hazards of cigarette smoking.

Major Findings: Compounds which are non-carcinogenic or weakly carcinogenic in most biological test systems have shown pronounced tumor initiating activity on mouse skin. In these experiments, a single dose of the initiating agent was applied on mouse skin (ICR/Ha Swiss) followed by repeated application of phorbol myristate acetate for one year or longer. The aromatic hydrocarbon dibenz[*a,c*]anthracene, sometimes quoted in the literature as "non-carcinogenic" and already shown to be carcinogenic by direct skin tests, was found here to act as initiator when tested in this manner. When tested as an initiating agent, another weak carcinogen, benz[*a*]anthracene, also resulted in significant tumor yields.

Two major findings have been that the compounds anthralin and safrole both have tumor promoting activity. Safrole is relatively weak but the anthralin has a promoting activity which is greater than any known compounds other than the phorbol esters. Safrole is related to the compound myristicin which has been isolated from tobacco tar.

An assay for promotion activity in cell culture has been developed based on an outgrowth of normally contact inhibited cells in a mixed cell culture.

Significance to Biomedical Research and the Program of the Institute: The identification and characterization of the factors in cigarette smoke are paramount requirements in our understanding of the process of lung tumorigenesis induced by cigarette smoke. The program is clearly designed to identify these factors so that they can be either removed from the cigarette or chemoprophylaxis developed to counteract their action.

Proposed Course: The fractionation of whole cigarette tar by the gentle non-destructive techniques will yield a new series of fractions that will be assayed for tumor-promoting potency using the initiation-promotion assay system. Using the 3T3 mouse cell line, the mixed culture assay system for tumor promoters will be further examined to determine the specificity of the method with regard to different types of tumor-promoting agents. The types of materials to be examined will include whole cigarette tar and fractions thereof. Tween 60, Span 80, anthralin, dimethylaminododecane (Penar), maleic hydrazide, acrolein and several long chain aliphatic hydrocarbons, aliphatic alcohols, and aliphatic acids. In addition, some phenols, coumarins and flavones earlier found by us to be inactive as tumor promoting agents will be tested in the mixed culture system, as "negative" controls. The biological specificity will be examined by an investigation of cell lines from other species such as rat and hamster. The important question of whether cells derived from primates, including human cells, will respond in this system will be studied.

Date Contract Initiated: June 29, 1964

Current Annual Level: $75,835

NEW YORK UNIVERSITY (NIH-71-2020)

Title: Alkylating Agents as Carcinogens and Anti-Carcinogens

Contractor's Project Director: Dr. Benjamin Van Duuren

Project Officer (NCI): Dr. Harry Gelboin

Objectives: Study of alkylating agents as carcinogens and anti-carcinogenic agents in cancer induction.

Major Findings: Past work on this contract has revealed the carcinogenic activity of several chloro ethers for mouse skin and subcutaneous tissue of mice and rats. These chloro ethers were then tested by inhalation in rats where they induced epidermoid carcinomas of the bronchi and lung. Since these chemicals are widely used in industry, a series of these compounds were used in tests which are underway or completed.

These studies included an examination of the carcinogenicity of isomers of epoxides and lactones. Thirteen compounds of this type of low molecular weight were tested for carcinogenic activity by lifetime subcutaneous injection in ICR/Ha Swiss mice. The compounds tested were epoxides, their nitrogen or sulfur analogs, a polymeric aldehyde, and an acetal. The compounds were selected for bioassay on the basis of their chemical reactivity and the relationship of chemical structure to carcinogenic activity. Two of these compounds, propane sultone (1-propanesulfonic-acid-3-hydroxy-γ-sultone) and aziridine ethanol (beta-hydroxy-1-ethylaziridine), induced sarcomas and other tumors at the injection site. Propane sultone resulted in tumors of the injection site in 21 out of 30 mice--twelve sarcomas, one carcinoma, seven adenocanthomas, and one papilloma. Aziridine ethanol administration resulted in sarcomas at the injection site in ten out of thirty mice. The following compounds induced a low incidence of tumors: 3,4-epoxysulfolane, 1,2-epoxybutyronitrile, 3,4-dihydroxy-3-cyclobutene-1,2-dione, ethyl-2,3-epoxybutyrate, tetramethyl-1,3-cyclobutanedione, perchloro-2-cyclobutene-1-one, and polymeric dialdehyde. The following compounds were inactive when injected subcutaneously: 3-sulfolene, 3,4-epoxybutanal diethyl-acetal, 5,6-epoxyhexanal diethylacetal, and N,N-dimethyl-formamide dimethylacetal.

Significance to Biomedical Research and the Program of the Institute: The future accomplishments of this group are expected to be in the realm of identifying the carcinogenic hazard of industrial chemicals. The identification and removal of these chemicals from the industrial environment is in the interest of the Government and cancer research.

Proposed Course: An additional series of chlorinated organic compounds will be tested. The compounds were selected on the basis of structure-carcinogenicity relationships and their wide commercial use. The compounds are as follows: 1-chloro-2-propanol; 2-chloro-1-propanol; 2,3-dihydroxychloropropane; chloroacetone; 2-chloro-N-methylacetamide; ethyl chloroacetate; ethyl bromoacetate; chloroacetic acid; bromoacetone; epichlorohydrin; 2,3-dichloro-p-dioxane; N-chlorosuccinimide; dimethylcarbamolyl chloride; and cloroacetonitrile. The compounds will be tested by topical application on mice and by subcutaneous injection in mice.

All compounds will be tested as commerical products but they will be thoroughly examined chemically for trace impurities. Chemical studies will be carried out on all compounds in order to determine chemical reactivity-carcinogenicity relationships. In these studies, the rates of hydrolysis of the compounds will be examined. Also the rates of reaction of chloromethyl methyl ether and bis(chloromethyl)ether, earlier shown to be carcinogenic, will be examined by this method. Where compounds are found to be carcinogenic, their reactivity towards nucleophiles and model compounds for tissue constituents will be examined and reaction products characterized by standard organic chemical techniques including elemental analysis, infra-red and nuclear magnetic resonance spectroscopy.

Date Contract Initiated: October 1, 1970 (This project was previously supported as Part II of New York University (PH43-66-962)

Current Annual Level: $90,481

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NIH-69-2144)

Title: The Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay Using Intratracheal Instillation Techniques in Syrian Hamsters

Contractor's Project Director: Dr. Robert Farrell

Project Officer (NCI): Dr. David Kaufman

Objectives: To determine effect of intratracheal instillation of fine particulates of varied chemical and physical properties and of various types of solvents on the induction of carcinoma of the lung by chemical carcinogens.

Major Findings: Chemically and physically defined fine particles, both coated with benzo[*a*]pyrene and uncoated, have been produced by another contract (IIT Research Institute--NIH-70-2245). Long term carcinogenesis studies are now in progress to determine and compare the carcinogenicity of these preparations. Preliminary evidence from a long term carcinogenesis experiment to determine whether Tween 80 is co-carcinogenic with benzo[*a*]-pyrene, has shown that this combination causes bronchiolar and alveolar epithelial hyperplasia and metaplasia. A bronchiolar adenocarcinoma has been found so far in an animal treated with this combination.

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observation that epidermoid carcinomas of the lung could be induced in hamsters by the intratracheal administration of iron oxide dust along with the intratracheal administration of benzo[*a*]pyrene. Further elaboration of these observations is needed to determine the role of atmospheric dusts or cigarette smoke particulates on the development of bronchogenic carcinoma in man.

Proposed Course: Several years will be required to complete long term carcinogenesis studies designed to determine the essential characteristics of particles coated with benzo[*a*]pyrene, and uncoated, as they affect the induction of epidermoid carcinoma of the lung. It is also proposed to examine the enhancement by chemically and physically defined particles of respiratory carcinogenesis by diethylnitrosamine.

Date Contract Initiated: June 26, 1969

Current Annual Level: $72,981

OREGON STATE UNIVERSITY (PH43-68-1462)

Title: Co-Carcinogenicity and Teratogenicity of Cyclopropenoid Compounds

Contractor's Project Director: Professor R. Sinnhuber

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To determine the possible co-carcinogenic effects of cyclopropenoid fatty acids (CFA) when fed to rats along with low doses of carcinogens (diethylnitrosamine [DEN], aflatoxin) to investigate the teratogenic effects of CFA, and to study the short-term distribution of CFA in rats.

Major Findings: At a level of 2% of *Sterculia foetida* oil in the diet of Wistar rats, the litter size, weight of young and one-week survival decreased from that of control rats or rats fed only 0.2% *S. foetida* oil. The diets containing both CFA and DEN led to a slightly higher incidence of hepatomas and metastases to the lung than did DEN alone.

Significance to Biomedical Research and the Program of the Institute: This project is related to other NCI-sponsored programs on the possible carcinogenic or co-carcinogenic action of materials present in foodstuffs. Although the levels of CFA in the food grade cottonseed oil in the United States are negligible, due to the processing now employed, cottonseed meal and some seed oils used in Japan contain considerable amounts of CFA.

Proposed Course: The project will end this year.

Date Contract Initiated: June 28, 1968

Current Annual Level: $39,986

UNIVERSITY OF SAN FRANCISCO (PH43-64-886)

Title: Studies of Carcinogenicity of Metallo-Organic Compounds

Contractor's Project Director: Dr. Arthur Furst

Project Officer (NCI): Dr. Richard Bates

Objectives: The purpose of this contract is the testing of metals and metallo-organic compounds for carcinogenicity by a variety of routes of administration in several species of animals, and the analysis of the process by which metals and their derivatives induce cancer.

Major Findings: Most of the earlier studies under this contract involved intramuscular injection of metals and metallo-organic compounds. These showed that nickelocene, nickel acetate, nickel powder, ferrocene, titanocene, and titanium dioxide induced local fibrosarcomas. In addition, titanocene and other titanium compounds induced lymphomas and leukemias. Other metals are now being tested by the same route of administration, and among these, cadmium also induces local sarcomas. More recent studies involve administration of these materials by other routes of administration, such as intratracheal and intragastric. Most of these experiments are too recent to have produced definitive results, but a small percentage of animals administered nickelocene by gastric intubation have developed leukemias and hepatomas.

Significance to Biomedical Research and the Program of the Institute: Metals and metal-containing compounds are widely used in industry and are extensively distributed through the environment. Thus, it is important to determine their carcinogenicity so that any potential human hazard can be minimized.

Proposed Course: The contractor is a major resource to the carcinogenesis program as an expert on the industrial usage of metals and their carcinogenicity. He will continue long-term studies along the lines outlined here of those metals to which humans receive significant exposure.

Date Contract Initiated: June 3, 1964

Current Annual Level: $47,010

SCIENTIFIC LITERATURE CORPORATION (NIH-70-2036)

Title: *Carcinogenesis Abstracts,* Volume VIII

Contractor's Project Director: Mr. S. Sim Kessler

Project Officer (NCI): Mr. Howard Rosenberg

Objectives: The publication of the abstract journal, *Carcinogenesis Abstracts,* provides an up-to-date abstract service for carcinogenesis researchers throughout the world. The format and content of the journal are designed to provide under one cover abstracts of published scientific work in the various disciplines which relate to cancer etiology.

Major Findings: All issues of *Carcinogenesis Abstracts,* Volume VIII, have been printed and distributed.

Significance to Biomedical Research and the Program of the Institute: The Etiology Area of NCI, as it is now constituted, is made up of both intramural and contract research efforts in chemical and viral carcinogenesis and in biometry and epidemiology. The various scientific disciplines which are involved in or related to these investigations are many and varied. A great variety of researchers throughout the world contribute to the study of cancer etiology. The extreme difficulty of interrelating these diverse disciplines and the numerous factors that are, to some degree, implicated in carcinogenesis require that various mechanisms of communication between scientists of related disciplines be identified and put into play. *Carcinogenesis Abstracts* has proven in the past to be a very useful and productive communication medium for both the researchers supported by NCI and others as well. A survey carried out on the use of and interest in this journal yielded a considerable positive response from working scientists. Investigators confirmed that the journal was valuable as an interdisciplinary alerting service as well as a vehicle through which to keep up with their own fields of endeavor.

Proposed Course: Present plans call for the continuation of this publication for an indefinite period of time. The Office of Management and Budget has approved publication and free distribution to qualified recipients through July 31, 1972. At that time the NCI will determine if application should be made for continuation of the journal. Periodic assessment of the abstracts for quality and relevance will continue to be made with the assistance of the Etiology staff. In addition, comments and recommendations for improvement of the publication will be solicited from other sources. Recent efforts have focused on bringing the publication up to date, so that it may serve optimally as a current awareness tool.

Date Contract Initiated: November 18, 1969

Current Annual Level: $46,740

SOUTHERN RESEARCH INSTITUTE (PH43-68-998)

Title: Determination of the Carcinogenicity of Cancer Chemotherapeutic Drugs and Other Agents

Contractor's Project Directors: Dr. F. M. Schabel
Dr. D. P. Griswold

Project Officer (NCI): Dr. John Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity of drugs used clinically or in pre-clinical stages, and of other compounds of comtemporary interest.

Major Findings: The study of the carcinogenicity of 47 single chemicals or combinations of agents, studied in Swiss mice and Sprague Dawley rats of both sexes at two dose levels has reached the stage of generation of results. Tumors of several types and at a variety of sites were noted, including adenoma and adenocarcinoma of the lung and breast, lymphosarcoma, leukemia and peritoneal fibrosarcoma with the following drugs: melphalen, chlorambucil, uracil mustard, natulan, dimethyltriazenoimidazole carboxamide, and 1,3-bis(2-chloroethyl) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (BCNU and CCNU). Animals on dibromo mannitol developed blindness.

In a related study, assessing the immunosuppressive potency of several of the drugs studied in the main program and involving the determination of heterologous tumor takes, showed that azothioprine (Imuran), and prednisone singly were not highly effective. However, combined with antilymphocyte serum, or the latter by itself, in most cases demonstrated immunosuppressive potency.

Significance to Biomedical Research and the Program of the Institute: There have been cases of patients treated with a drug for Hodgkins' disease and other types of neoplastic disease who were placed in remission. Later, new primary growths attributable to the antecedent course of drugs arose. Physicians and the public need guidance on the possible consequences of utilizing certain drugs and chemicals, so they can weight the risk-benefit ratio and hopefully not only cure but also prevent disease. Because of high contemporary interest, a paper reporting these results will be presented at the 1971 American Association for Cancer Research meeting.

Proposed Course: The program now is in the middle of the large-scale autopsy series which will be followed by extensive histopathologic study. This main effort will therefore continue through 1972. Furthermore, in order to perform studies of this type most efficiently, programs will be developed to phase in drugs and other chemicals of interest on a more even basis over a period of time so that the subsequent conduct of the extensive studies including the final histopathologic evaluation can be arranged with a more regularly spaced work load.

Date Contract Initiated: June 24, 1968

Current Annual Level: $219,494

SOUTHWEST RESEARCH INSTITUTE (PH43-65-1000)

Title: Free Radicals and Alkylating Agents in Tobacco Smoke

Contractor's Project Director: Dr. Donald Johnson

Project Officer (NCI): Dr. John Cooper

Objectives: The principal objective of the contract is the development and application of methodology for the analysis of N-nitrosamines. More specifically, the emphasis has been placed on tobacco smoke as a potential source of N-nitrosamines. The overall objective of the contract is to identify and measure those compounds which contribute to the total carcinogenic activity of tobacco smoke. The method should also be useful for application to other samples such as food products.

Major Findings: The method which was completed in this past support period was applied to the analysis of cigarettes made from several different types of tobacco. The results indicated that dimethylnitrosamine was present in the smoke from these cigarettes and the level varied with the type of tobacco. The data suggest that the type of field treatment that tobacco receives could drastically affect the amounts of this carcinogen. The identity of the peak believed to be dimethylnitrosamine was not unequivocally established and is now under additional study.

Significance to Biomedical Research and the Program of the Institute: Analytical studies of tobacco smoke constitute an essential component of carcinogenesis studies directed toward the identification of cancer hazards related to tobacco smoke. In view of the importance of nitrosamines as a carcinogenic biohazard, the analytical results obtained by the present group are essential for overall program development.

Proposed Course: Emphasis during the first part of present support period will be on the identification of the unknown compound tentatively identified as dimethylnitrosamine. If the identity of this compound is confirmed, an in-depth study will be initiated to determine the effects of various tobacco smoking parameters on the amounts of nitrosamines in the smoke condensate.

Date Contract Initiated: June 23, 1965

Current Annual Level: $41,600

STANFORD RESEARCH INSTITUTE (NIH-71-2045)

Title: Retrieval and Ranking of Potential Carcinogenic Hazards

Contractor's Project Director: Mr. Arthur McGee

Project Officer (NCI): Dr. Gio Gori

Objectives: Define and demonstrate the feasibility of a systematic methodology for information retrieval leading to the ranking of environmental chemicals for carcinogenesis testing. This selection will be based on the estimated risk for potential chemical carcinogenic hazards to man.

Major Findings: Contract activities were initiated in November of 1970. The project was organized into four major task groups: environmental exposure, chemical activity, information processing and system integration.

The environmental exposure team has developed a general methodology for determining and quantifying the exposure of humans to chemical compounds. This methodology includes the subdivision of exposure into well-defined subcategories that relate to readily available data sources and expert opinion, thus facilitating analysis and use of the data. The methodology has been applied on a test basis to household soaps and detergents. The team is proceeding to apply the methodology to food additives and air pollutants.

The chemical activity team has developed methodology that enables us to relate suspected carcinogenic activity to chemical structure. In support of the methodology, a qualitative analysis has been made of the "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" (PHS Publication No. 149). The methodology has also been developed in depth for several chemical groups using to some degree the services of outside consultants. A parallel effort is underway investigating the use of information on related biological phenomena (i.e. mutagenic, teratogenic and immunosuppressive compounds) as a criteria for suspicion of carcinogenic activity.

The information processing team has identified readily available data sources of potential use in the methodology, both in manual and machine readable form. The feasibility of using a computer program for assigning the probability of suspicion based on chemical structure is being investigated and tested using data from SRI's Chemical Economics Handbook and Directory of Chemical Producers.

The systems integration team has developed a preliminary procedure for arriving at a rank list of compounds for bioassay based upon environmental exposure and chemical activity criteria. A preliminary test of this methodology is underway and seems promising at this time.

Significance to Biomedical Research and the Program of the Institute: The system developed through this study will be useable in identifying and ranking those chemical compounds that pose the greatest suspected hazard to humans for use in guiding NCI's carcinogenic bioassay program. The methodology will represent the best knowledge available within the scientific community.

Possible by-products of the study will be to identify areas where additional information regarding chemical activity of human exposure will enhance the effectiveness of the bioassay testing program.

Proposed Course: To continue the project as outlined above or as recommended by the project officer.

Date Contract Initiated: October 30, 1970

Current Annual Level: $309,455

TEMPLE UNIVERSITY (PH43-64-504) (Formerly: University of Pittsburgh)

Title: Research on the Biochemical and Morphological Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. Emmanuel Farber

Project Officer (NCI): Dr. Michael Sporn

Objectives: To define the correlation of morphological, biochemical and biologic alterations observed in the liver during hepatic carcinogenesis.

Major Findings: Large hyperplastic nodules can be induced in over 60% of male highly inbred Fischer rats fed aflatoxin B_1 in the diet at a level of 3 parts per million (ppm). The nodules induced by aflatoxin resemble those induced by 2-fluorenylacetamide (2-FAA) or ethionine in that they show the following characteristics: (a) retain glycogen on fasting in contrast to the surrounding liver, (b) show progressive decrease in glucose-6-phosphatase activity, (c) have abundant smooth endoplasmic reticulum (ER) with diminished amounts of rough ER, (d) trypsinized suspensions of cells from hyperplastic nodules reproducibly grow in tissue culture under conditions in which the normal or surrounding liver does not. The growth characteristics of the nodular cell population are different than those of liver cancer in that they only grow initially on plastic and not on glass and they grow only for six to eight generations and then stop. When grown on collagen-coated sponge, they show differentiation to islands resembling liver parenchyma interspersed with ducts. The cells appeared to be hepatocytes both by their appearance with light and electron microscopy and by their ability to make rat serum albumin.

Thus, it is now apparent that with three different hepatic carcinogens, aflatoxin B_1, 2-FAA and ethionine, the cells of the hyperplastic nodules have very similar properties, a conclusion that may begin to be exploited in the development of a much more rapid assay for hepatocarcinogenicity of unknown compounds.

Significance to Biomedical Research and the Program of the Institute: Hyperplastic nodules are a critical and potentially requisite, precancerous lesion which occurs prior to overt carcinomas. It is desirable to have as much biochemical and morphologic information as can be obtained on the events that characterize the early cellular response to carcinogens. These techniques may in the future become useful in screening procedures of suspected carcinogens.

Proposed Course: To continue to define morphological and biochemical correlations in hepatic hyperplastic nodules produced by carcinogens, with the aim of elucidating common cellular and molecular changes caused by these carcinogens.

Date Contract Initiated: January 20, 1964

Current Annual Level: $32,000

TEMPLE UNIVERSITY (PH43-65-1029)

Title: A Search for Carcinogens among Mycotoxins

Contractor's Project Director: Dr. Fritz Blank

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: This program is aimed at identification of possible carcinogens from fungi pathogenic to man, especially the *Candida* species. The original program involved a screening of various fractions and extracts from 17 fungal species. Presently, efforts are being concentrated on *Candida parapsilosis, C. albicans,* and *Scopulariopsis brevicaulis* since the preliminary results showed these to be most suspicious.

Major Findings: Mycelia of the fungi are produced and dried (Dr. Blank). Extracts of the mycelia (hexane, chloroform, hot and cold methanol) are prepared both for bioassay and chemical fractionation (Dr. G. Just, McGill University). Bioassays are performed by Dr. S. Mann at Temple University. Repeated injection of CFW mice with lyophilized mycelia of *C. parapsilosis* in tricaprylin led to a striking increase in the leukemia incidence, but hot and cold methanol extracts of the mycelia had only a weak effect in producing tumors. In strain A mice, injection of a high dose of the hot methanol extract of *C. parapsilosis* gave a significant increase in frequency of lung tumors. Liquid chromatography of the various extracts is being developed.

Significance to Biomedical Research and the Program of the Institute: This study deals with potential carcinogenic fungal metabolites which may be of importance to man, through contact on skin and hair or through their presence on foods or other commodities.

This program is related to other studies on mycotoxins such as those of the Massachusetts Institute of Technology (PH43-62-468) and the City of Hope (PH43-65-1036) program.

Proposed Course: This project will continue with emphasis on determining the active constituents in the fungi. Development of liquid chromatographic system may enable correlation of the bioassay and chromatographic results.

Date Contract Initiated: June 12, 1965

Current Annual Level: $79,120

UNIVERSITY OF TENNESSEE (NIH-69-2077)

Title: Carcinogenic Studies of Polyurethanes

Contractor's Project Director: Dr. John Autian

Project Officer (NCI): Dr. Richard Bates

Objectives: There have been a few reports in the past indicating that some polyurethane plastics may be carcinogenic. However, these plastics have widely diverse chemical structure, and the structure of those inducing tumors has not been known. The objective of this project is to test a variety of polyurethanes of known chemical composition by intraperitoneal implantation in rodents in order to search for a relationship between carcinogenicity and chemical structure of these plastics.

Major Findings: It is too early to assess the results of this project. Tumors have just begun to appear in the experimental animals. So far, these are principally fibrosarcomas which could be induced by the physical nature of the implants rather than by their chemical composition. It is anticipated that the bulk of the information from this contract will be produced during the coming year.

Significance to Biomedical Research and the Program of the Institute: Polyurethane plastics are an extraordinarily diversified group of materials which have a very widespread and growing usage. Commonly used polyurethanes are essentially co-polymers of aromatic amines and carbamates; both of these classes of compounds include carcinogenic members. This fact, plus the previous observation of tumor induction by polyurethanes stresses the need for study of these materials. The most likely hazard to man comes from inhalation of polyurethane dusts during the fabrication process. However, because of the high expense of inhalation studies, initial experiments to define the relationship between chemical structure and carcinogenicity are being performed by intraperitoneal implantation.

Proposed Course: Implantation of the plastics is complete and the animals will be followed for the remainder of their lifespan which in most cases should amount to approximately one to one and one-half years.

Date Contract Initiated: June 23, 1969

Current Annual Level: $74,000

THOMPSON, JOHN I. AND COMPANY (NIH-69-2086)

Title: Literature Search and Retrieval of Specific Data Relating to Chronic Tests of Chemicals in Experimental Animals

Contractor's Project Director: Mr. John King

Project Officer (NCI): Mr. Howard Rosenberg

Objectives: This project provides for compilation and publication of data from the 1961-1969 scientific literature on effects of chemicals in experimental animals. Resultant publications comprise supplements of PHS Publication No. 149, formerly entitled *Compounds Which Have Been Tested for Carcinogenic Activity.*

Major Findings: Two supplements, covering the literature from 1961-1964 and 1968-1969 respectively, are in press and will be distributed within a few months. Preparation of another, covering 1965-1967, is nearly completed.

Significance to Biomedical Research and the Program of the Institute: Consistent with the structure and goal orientation of research supported by the Carcinogenesis Program, there is constant need for documentation of work in the field. PHS Publication No. 149 is a valuable reference that serves in not only the dissemination of scientific information but also the avoidance of research duplication. Response to initial issues of the series and more importantly to the absence of supplements covering 1961-1969 has underlined the acute need for this tool.

Proposed Course: The work of this contract should be completed within a few months with a 1965-1967 supplement going to press. The series will be extended to include a 1970-1971 supplement, which will be prepared under a new contract.

Date Contract Initiated: June 9, 1969

Current Annual Level: $156,350

WEIZMANN INSTITUTE (NIH-70-2217)

Title: Study of the Role of Enzyme Induction in Chemical Carcinogenesis

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry Gelboin

Objectives: Using the *in vitro* methods for the induction of neoplastic transformation by chemicals, developed by Dr. Sachs, this project has the following objectives: (1) to study the relationship of aryl hydrocarbon hydroxylase activity to toxicity and transformation by chemical carcinogens *in vitro*; (2) to study the effect of enzyme inducers and inhibitors on cell transformation in normal and revertant cells; and (3) to study the

the properties of revertant cells and the conditions favoring carcinogenic transformation and reversion.

Major Findings: (1) Studies on the level of aryl hydrocarbon hydroxylase in various types of cells have shown a correlation between the level of enzyme activity and the susceptibility of cells to the cytotoxicity produced by benzo[*a*]pyrene. These results have indicated that aryl hydrocarbon hydroxylase is the enzyme system responsible for cell susceptibility to the cytotoxic effect of benzo[*a*]pyrene and that the toxic effect of this carcinogen is due to its enzymatic conversion to a cytotoxic metabolite. An inhibitor of aryl hydrocarbon hydroxylase, 7,8-benzoflavone, also inhibits the cytotoxicity of benzo[*a*]pyrene. Results of experiments of cell transformation of hamster embryo cells have indicated that this inhibitor of enzyme activity inhibits cell transformation. This suggests the metabolism of benzo[*a*]pyrene by aryl hydrocarbon hydroxylase is required for cell transformation as well as for cytotoxicity.

(2) Cells transformed by benzo[*a*]pyrene and dimethylnitrosamine, in which the transformed state has become a hereditary cellular property, display a high frequency of variants with a reversion of properties of transformed cells. These variants lacked the random crisscross pattern of growth shown by the parental transformed cells, and they had regained their contact inhibition of cell replication with the same specificity as that found with normal cells. Compared to the parental transformed cells, these variants also showed a decrease in tumorigenicity, saturation density, and cloning efficiency in liquid medium and in soft agar. In contrast to such variants from polyoma virus transformed cells, 81-93% of the variants from the cells transformed by the chemical carcinogens had again, like normal cells, acquired a limited lifespan *in vitro*. This termination of lifespan was intracellularly determined. The escape from the limited lifespan by some of these variants was due to a re-reversion from the reverted to the transformed state. The results have shown that cells transformed by these two chemical carcinogens can completely revert back to normal cells.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons represent major contaminants in the environment and are serious threats to the human organism in a highly industrialized society. The enzyme system dealing with these agents is aryl hydrocarbon hydroxylase, and our findings indicate that the enzyme system is responsible for the activation of the hydrocarbon to a toxic and probably a carcinogenic form. The observation that transformed cells can revert back to normal properties is an exciting and important finding that may enable an understanding of conditions favoring a reversion of carcinogen transformed cells back to a normal state.

Proposed Course: To further clarify the relationship between the presence of the enzyme, the metabolism of carcinogenic hydrocarbons, and malignant cell transformation. Inhibitors of the enzyme, such as 7,8-benzoflavone, will be tested for their effect on cell transformation induced by carcinogenic hydrocarbons other than benzo[*a*]pyrene. The revertants produced from transformed cells without detectable enzymes will be tested for their enzyme activity in order to determine whether the suppression of malignancy results in the reappearance of the enzyme in the reverted cells. Revertants from cells transformed by carcinogenic hydrocarbons and other chemical carcinogens will also be tested for their susceptibility to the cytotoxic and cell transforming activity of carcinogenic hydrocarbons. Cells transformed by polyoma virus exhibit a reversion which is controlled by the balance between two types of chromosomes, one of which determines the expression of malignancy and the other its suppression. Cells transformed by chemical carcinogens and their revertants will be examined for their chromosome constitution, in order to determine to what extent their chromosome balance determines the expression and suppression of malignancy in these cells and which chromosomes determine the presence of the hydroxylase enzyme system.

Date Contract Initiated: May 26, 1970

Current Annual Level: $51,000

LUNG CANCER TASK FORCE
TOBACCO PROGRAM

AMERICAN HEALTH FOUNDATION (NIH-70-2087)

Title: Evaluation of Carcinogenic Agents in Cigarette Smoke; Biological and Chemical Assays and Epidemiological Studies

Contractor's Project Directors: Dr. Ernest Wynder
Dr. Dietrich Hoffman
Dr. Po Chan

Project Officer (NCI): Dr. Gio Gori

Objectives: Investigation of the relationships of smoking and health. Laboratory investigations include chemical analyses and bioassays of cigarette smoke condensates and bioassay methodology, aimed at the definition of hazards in smoke, and on the development of a less hazardous cigarette. Epidemiological studies are aimed at identifying means of disease prevention by definition of less hazardous products and smoking habits, and by identification of susceptible subjects and less favorable environmental conditions. A supplemental program is aimed at establishing basic information on the carcinogenicity of marijuana by a combined chemical and biological investigation.

Major Findings: Epidemiological studies - preliminary findings show a continuing trend for a reduced lung cancer risk among individuals who have smoked filtered cigarettes for long periods. Studies of all tobacco related cancers are in progress in New York, Houston, and Los Angeles. Biological work to date indicates a lower complete carcinogenicity for tars from reconstituted tobacco sheets tested on mouse skin, but not a reduction of tumor promoting activity. All other biological experiments are still in progress. Chemical analysis of the various tobacco tars are also in progress.

Significance to Biomedical Research and the Program of the Institute: The NCI has major responsibility in the definition of smoking hazards. Scientific expertise in this field is of vital importance in the current programs of the Lung Cancer Task Force at the NCI. Resources capable and willing to perform biochemical and epidemiological surveys in this field are extremely limited. Therefore, the outstanding position of the American Health Foundation fulfills an essential need of the NCI. Additional opportunities for expansion of scientific efforts by this contractor have been identified, should the funding situation improve.

Proposed Course: To continue as outlined above.

Date Contract Initiated: February 24, 1970

Current Annual Level: $258,175

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (40-117-67)

Title: Collection, Separation, and Elucidation of the Components of Cigarette Smoke

Contractor's Project Directors: Dr. Michael Guerin
Dr. Wilbur Shults

Project Officer (NCI): Dr. Gio Gori

Objectives: To establish a facility for the chemical characterization of cigarette smokes and make that facility available to NCI approved contractors. To apply state of the art analytical methodologies for a more definitive chemical characterization of smokes. To provide chemical data characterizing experimental cigarettes developed in the evolution of a less hazardous cigarette.

Major Findings: The core of the routine facility is established and is being applied to a series of 23 NCI provided experimental cigarettes. Smoke particulate matter, gas phase, and condensate are being characterized. Parameters measured include total particulate matter, water, nicotine, nornicotine, tar, hydrogen cyanide, formaldehyde, acetaldehyde, acrolein, oxides of nitrogen, pH, acids, phenol, o-cresol, m-cresol, p-cresol, phenanthrene, benzo[*a*]pyrene and benz[*a*]anthracene. Novel analytical methods have been considered which pose considerable promise in improving the routine methods, expanding the number of species conveniently assayed, and more fully characterizing smokes.

Significance to Biomedical Research and the Program of the Institute: The NCI has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and the steps which might be taken to produce a non-carcinogenic cigarette. Chemical and biological research toward these objectives have suffered from the lack of an adequate characterization of the cigarette smokes studied. This facility provides that needed characterization. Results are generated in such a way as to allow a given cigarette to be compared to a series of highly characterized cigarettes. The data is of major significance in designing and controlling experiments as well as in interpreting the results of bioassays and product modifications. The routine facility provides a vehicle for making state of the art methodologies available to any qualified contractor. The extensive analytical chemical capabilities at this laboratory allow essentially any smoke matrix or component to be studied. The cooperation of industry laboratories provides a new dimension of significance to the generated results.

Proposed Course: To continue as outlined above. To incorporate considerations of whole smokes, particularly as generated for inhalation bioassay, in the chemical characterization facility.

Date Contract Initiated: November 1969

Current Annual Level: $100,000

HAZLETON LABORATORIES, INC. (NIH-69-2149)

Title: Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice

Contractor's Project Director: Mr. James Gargus

Project Officer (NCI): Dr. Gio Gori

Objectives: The objective of this project is to provide a standardized bioassay for determination of the carcinogenicity of cigarette smoke condensates by repeated epidermal applications in mice.

A total of 29 different whole smoke condensates are being investigated in this program. The coded condensates are supplied by NCI from Meloy Laboratories. The variables in these condensates include tobacco of different origin, methods of cultivation, including fertilization, pesticides, suckering agents, soils; curing and aging methods, blends of tobacco plant parts such as leaves, stalks, stems; reconstituted tobacco, cutting of tobacco, cigarette paper and cigarette filters.

Female ICR mice are treated six times weekly with epidermal applications of the whole smoke condensates dissolved in acetone. Skin tumors are recorded and measured. All animals are necropsied and tissues are fixed and preserved for histopathological examination. Data on each animal are entered in a computer memory once monthly. Computations and printed reports from NCI provide monitoring data for continuous evaluation of the experimental results.

Major Findings: Epidermal applications were initiated in April 1970. A total of 26 condensate variables were placed on study during April and May of 1970. In December 1970 three additional experimental variables and appropriate controls were initiated. High level (100 mg) applications three times weekly resulted in excessive mortality in many experimental groups. This was resolved by increasing the frequency to six applications weekly and lowering the maximum dose. The levels of application were established as 25 mg for the low level groups and 50 mg per application for the high level groups. Monthly animal data are submitted for computer storage and computations. All tests are still underway.

Significance to Biomedical Research and the Program of the Institute: The objective of this project is to provide a standardized bioassay for determination of carcinogenicity of cigarette smoke condensates by dermal application to mice. These comparative bioassay results along with other monitoring bioassays and chemical determinations will permit evaluation of the biological effects of the variables in the different smoke condensates. These comparative evaluations will provide data for testing and developing less hazardous cigarette formulations.

Proposed Course: To continue as outlined above.

Date Contract Initiated: June 24, 1969

Current Annual Level: $256,250

HAZLETON LABORATORIES, INC. (NIH-69-2145)

Title: Carcinogenicity Bioassay by Intragastric Intubation of Cigarette Smoke Condensates in Experimental Animals

Contractor's Project Director: Mr. James Gargus

Project Officer (NCI): Dr. John Cooper

Objectives: The objective of this activity is the evaluation of the carcinogenic potential of cigarette smoke condensates following repeated oral administration in rodents.

Experimental variables in the cigarette smoke condensates will include nicotine content and total dose. Once standardization is attained, the bioassay will be used for determining the carcinogenic potential of smoke condensate from modified cigarettes, as a part of the monitoring system in the program for the development of a less hazardous cigarette.

Major Findings: A two-week dose range study in rats and mice was performed to obtain necessary data on levels of administration in the subacute study. A three-month subacute study was initiated in July 1970 and included two strains of mice, two strains of rats and hamsters. The objective of this study was to provide toxicological data on the effects of smoke condensates and to determine the two species of rodents to be used in the 18-month chronic study. The long-term feeding study will be initiated in May 1971.

Significance to Biomedical Research and the Program of the Institute: This project is an integral part of the program for the formulation of a less hazardous cigarette. This program requires that new bioassay techniques be devised which will be both sensitive and specific. The selection of this route of administration for emphasis is justified by the large quantity of tobacco tars and condensate which are ingested into the stomach in the course of smoking and subsequent ciliary clearance of the respiratory tract.

Proposed Course: Long-term feeding studies will be initiated in two animal species in the coming support period and will be continued for the lifetime of the animals.

Date Contract Initiated: June 24, 1969

Current Annual Level: $87,200

LITTLE, ARTHUR D. (NIH-69-2147)

Title: Bioassay of the Cytotoxicity of Cigarette Smoke and of Its Effects on Ciliary Function

Contractor's Project Director: Dr. Philip Thayer

Project Officer (NCI): Dr. Gio Gori

Objectives: After the short-range objectives of standardization of techniques and experimental models had been achieved, the longer range objectives of this project have been to evaluate the experimental cigarettes, provided by the program for development of a less hazardous cigarette. The methods developed and evaluations performed are for the following: (1) effects of smoke on cytotoxicity and phagocytic activity on *in vitro* cultivated cells; (2) effects of smoke on ciliary kinetics and dynamics of mucus transport in the respiratory epithelium of experimental animals *in vitro* and *in vivo*.

Major Findings: After preparatory efforts in the standardization of techniques, a complete set of assays on all experimental cigarettes has been carried out. All experiments will be reviewed before attempting analysis of their significance.

Significance to Biomedical Research and the Program of the Institute: The overall program for the formulation of a less hazardous cigarette needs to identify physiological responses of the respiratory tract surface tissues to cigarette smoke of different characteristics.

This information will be correlated with carcinogenesis data in testing and directing modification of cigarettes towards less hazardous formulations.

Proposed Course: Activities on this project were started in February 1970 when experimental cigarettes became available. A first series of evaluations of 23 types of experimental cigarettes in the four assays (cilia *in vivo*, cilia *in vitro*, cytotoxicity, and phagocytic activity) was performed in 1970. A second series was started, and is continuing as above.

Date Contract Initiated: June 19, 1969

Current Annual Level: $48,338

MELOY LABORATORIES (NIH-69-2084)

Title: Preparation and Analysis of Cigarette Smoke Condensate Samples

Contractor's Project Director: Dr. A. R. Patel

Project Officer (NCI): Dr. Gio Gori

Objectives: During one year period Meloy Laboratories will smoke over 4,000,000 cigarettes to produce the smoke condensate having, as nearly as possible, uniform composition for use in a number of bioassay programs to be carried out under contract in the Chemical Carcinogenesis/Lung Cancer Task Force Tobacco Program.

(1) For each of the 21 types of cigarettes (Codes 11 and 12 cigarettes were dropped from original list of 23, due to toxicity) the cigarettes are conditioned 75 ± 2°F and 60 ± 5% relative humidity, and smoked on two manifold type smoking machines furnished by the National Cancer Institute. The acetone extract of smoke condensate is concentrated under reduced pressure, and the residue is adjusted by the addition of acetone and water (6%) to the composition, 500 mg tar per ml of condensate. Condensates are shipped weekly to contractors according to the instructions of the NCI Project Officer. At least 420 gms of dry condensate is produced from each of the 21 types of cigarettes for an eight week skin painting study. During this year, Meloy Laboratories will also produce 60 gms of dry condensate from each of the 21 types of cigarettes for analytical chemistry at Oak Ridge National Laboratory, and 400 gms of condensate from codes 2 and 5 cigarettes, every 8 weeks for intragastric intubation studies.

Meloy Laboratories plans to make 2 million cigarettes from the marijuana furnished by the NCI: if everything progresses according to schedule, these cigarettes should be ready by the end of March 1971. They will be smoked on the manifold type smoking machine and the condensate collected in the usual manner, to be used in carcinogenesis studies by skin painting in mice.

(2) Meloy Laboratories carries out a quality control program of chemical analysis of all batches of cigarette smoke condensate both to insure uniformity and to characterize the product to some extent for the subsequent users. The following information is recorded on each batch: (a) mean butt length, (b) total condensate yield and condensate yield per cigarette, (c) moisture content, (d) acetone content, (e) condensate pH, (f) nicotine content of the condensate and nicotine content per cigarette.

(3) During the one year period Meloy Laboratories will carry out the following analysis on each of the 21 types of cigarettes: (a) nicotine in tobacco, (b) nitrate in tobacco, (c) sugars in tobacco, (d) ash, (e) hexane solubles, (f) potassium and sodium, (g) calcium and magnesium, (h) phosphorous, (i) static burning rate of cigarettes, (j) average temperature of firezone at different butt lengths, (k) tetrahydrocannabinols (for marijuana cigarettes only).

Major Findings: This is a complex service contract, the scope of which is production of specific materials. This production has been made with an outstanding quality control record.

Significance to Biomedical Record and the Program of the Institute: This contract is designed to produce the cigarette smoke condensate required for the execution of at least four other projects which will be funded under the Lung Cancer Task Force: (1) Skin Carcinogenesis Bioassay of Cigaratte Smoke Condensate, (2) Intratracheal Bioassay of Cigarette Smoke Condensate for Carcinogenesis, (3) Bioassay of Cigarette Smoke Condensate by Oral and Parenteral Routes, (4) Skin Carcinogenesis Bioassay of Marijuana Cigarette Smoke Condensate.

Proposed Course: Activities of this contract started in February 1970, as experimental cigarettes became available. Increased load of analytical activities is expected. Production activities are proceeding on schedule.

Date Contract Initiated: June 30, 1969

Current Annual Level: $263,725

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR
FOR VIRAL ONCOLOGY (OASDVO)

July 1, 1970 - June 30, 1971

J. B. Moloney

The Annual Report for Viral Oncology, Etiology, NCI, is presented as follows:

I. Viral Oncology Area - Special Virus Cancer Program (SVCP)

- A. Introduction
- B. Organization
  - 1. Viral Oncology Area
  - 2. Special Virus Cancer Program (SVCP)
- C. Scientific Activities - Progress Highlights
- D. Projections
- E. Publications - SVCP

II. Summary Reports

- A. Offices of the Associate Scientific Director for Viral Oncology
  - 1. Office of Biohazards and Environmental Control
  - 2. Office of Program Analysis and Communications
  - 3. Office of the Coordinator for Ultrastructural Studies

- B. Branch Reports
  - 1. Viral Leukemia and Lymphoma Branch
    - (a) Summary
    - (b) Individual Project Reports

  - 2. Viral Biology Branch
    - (a) Summary
    - (b) Individual Project Reports

  - 3. Viral Carcinogenesis Branch
    - (a) Summary
    - (b) Individual Project Reports

III. Special Virus Cancer Program Contract Summaries by Segments

I. VIRAL ONCOLOGY AREA - SPECIAL VIRUS CANCER PROGRAM (SVCP)

A. Introduction:

The Viral Oncology Area is responsible for planning and conducting the Institute's program of coordinated research on viruses as etiological agents of cancer. Scientists within this Area not only provide the broad operational management for intramural and collaborative research but also conduct comprehensive investigations on specific animal oncogenic viruses and their interaction with host cells and apply this information to search for viruses which may be etiologically related to the initiation and continuation of human cancer.

Contract supported research is conducted within the Viral Oncology Program under the Special Virus Cancer Program (SVCP) whose primary objectives are: (1) to determine whether viruses comparable to those known to induce cancers of laboratory and domestic animals are causative agents of human cancers, and (2) to develop therapeutic and preventive measures for control of human cancers when such causative agents are found. A detailed history of events leading to the development of the SVCP may be found in previous Annual Reports of the NCI. Briefly, in 1964, the Congress of the United States provided funds to the NCI for an intensified program in virus-leukemia research because many scientists were convinced that an effort to identify viruses or to detect virus expression in human tumors would contribute to the determination of the etiology of cancer. Using a new planning approach (Convergence Technique), an overall program aimed at controlling human leukemia and lymphoma was formulated. It is based on the premise that one virus is an indispensable element for the induction (directly or indirectly) of at least one kind of human cancer and that the virus or viral genome persists in the diseased individual. In 1968, the program was enlarged to encompass all types of cancer. The Program plan has undergone considerable revisions since its inception and has been reviewed regularly by the Director, NIH; Director, NCI; the National Cancer Advisory Council; the Scientific Directorate, NCI; and the Etiology Program Management Group, NCI.

During the past seven years, the Institute has developed an effective management program which has made rapid, substantial progress in understanding cancer induction by viruses. The funding level for this Program in fiscal year 1971 has been about $35 million.

B. Organization:

1) Viral Oncology Area. During this reporting period the area was reorganized in a structure that will permit the most effective use of scientists and facilities in the attainment of Program goals. All changes were implemented with existing personnel, space, and funds. By increasing the number of sections within the present three branches, additional positions of authority and responsibility were created.

On February 1, 1971 the Deputy Director for Science approved the NCI request to establish three offices and to redefine the functions of existing branches in the Office of the Associate Scientific Director for Viral Oncology. The Office of the Associate Scientific Director for Viral Oncology is organized as follows:

Office of the Associate Scientific Director for Viral Oncology

Plans and conducts the Institute's program of research and development dealing with viruses as etiological agents of cancer. Supports programmatic investigations aimed at the detection, propagation, characterization, prevention, and control of tumor viruses and/or their induced diseases.

Office of the Coordinator for Ultrastructural Studies
Immediate Office of the Coordinator
Virus Studies Section

Plans and conducts research on the morphology, ultrastructure, biological characterization and correlation of structure and function of viral agents associated with neoplasms of man and animals.

Office of Biohazards and Environmental Control
Immediate Office of the Chief
Biohazards Research Section
Environmental Control Section

Performs research in virology, aerobiology, mammalian physiology and biochemistry in order to evaluate the risk involved to the host under stress when exposed to infectious agents. Develops and recommends equipment and procedures for handling of potentially biohazardous materials and disseminates this information to the scientific community and to research laboratories throughout the world.

Office of Program Analysis and Communications

Manages computerized systems of program statistics, clinical and laboratory data interchange, and scientific information for collaborative studies with inhouse and contract scientists; applies computer techniques to data systems and retrieval problems of inhouse and contract laboratories; plans and conducts surveys; plans, implements, and maintains a controlled central data system and inventory for a multi-institutional human serum bank. Maintains a system of triannual progress reporting from research contracts; compiles and distributes summaries of current progress to program scientists. Maintains an automated system for publication searches and reference printouts on requested subjects in viral oncology; prepares a monthly publication featuring abstracts of current worldwide literature on viral oncology; compiles and publishes semiannually indexed summaries of current research in viral oncology; maintains technical reference library.

Viral Biology Branch (Office of the Chief)

Cell Biology Section
Electron Microscopy Section
Microbiology Section
Experimental Pathology Section
Human Tumor Studies Section
Virus and Disease Modification Section

Plans and conducts research to determine the relationship of viruses to neoplastic diseases of man and animals. Devises and tests effective means for the prevention and control of these diseases and viruses.

Viral Leukemia and Lymphoma Branch (Office of the Chief)

Tumor Virus Section
Immunology Section
Viral Pathology Section
Genetics Section
Molecular Biology Section

Plans and conducts research on virus-host relationships in leukemia and lymphoma with tumor viruses of RNA and DNA composition, with particular emphasis on molecular aspects of viral oncogenesis.

Viral Carcinogenesis Branch (Office of the Chief)

Ecology and Epizoology Section
Solid Tumor-Virus Section
Viral Genetics Section

Plans, conducts and manages research and development on the virology of animal and human neoplasms with emphasis on the natural history of tumors and their inducing agents.

2) Special Virus Cancer Program.
The Special Virus Cancer Program is composed of 7 working segments; i.e. Developmental Research Segment; Immunology Group; Program Resources and Logistics; Special Animal Leukemia Ecology Studies; Biohazards Control and Containment; Human Leukemia Therapy; and Solid Tumor Virus.

On February 1, 1971 the SVCP added an additional segment. This group, the Breast Cancer Virus Studies Segment is under the direction of Dr. W. R. Bryan, Chairman, and Dr. R. Depue, Vice-Chairman. The rationale for establishing this new segment is as follows: since the initiation of the Special Virus Cancer Program, exploratory studies based on information and leads from the mouse-mammary tumor virus (MTV) system have been conducted to determine whether evidence could be found for an association of viruses with breast cancer of humans or of animals other than mice, the only species, thus far, in which breast cancer is known to be caused by a virus.

The similarity of the "clustering" of breast cancer within human family groups to the "clustering" that had been observed for breast cancers of mice at the turn of the century, before the development of inbred mouse strains, suggested that a virus comparable to the mouse MTV might be involved in human disease. Since the MTV was not discovered until high breast cancer strains of mice had been developed by selective inbreeding, human population groups which would most closely approximate the experimental mouse models were selected for study. These included: (1) women of the relatively highly inbred Parsi sect of Bombay, India; (2) women of this country having a history of "family clustering" of breast cancer, e.g. among the mothers, sisters, maternal grandmothers, and maternal aunts of probands to be studied; and (3) women who actually have had breast cancer, but who have survived after successful treatment by removal of one breast. Other leads from the mouse model involved the use of milk and of tissue culture-propagated breast cancer tissue, as the specimens most likely to contain sufficient amounts of an etiologically related virus for detection of electron microscopy (the only method available for the detection of a human agent at this time).

The results of the human studies accumulated through this year provide substantial electron microscopic evidence of virus-like particles resembling the RNA tumor viruses of animals (both B- and C-type particles have been observed) in the milk of women belonging to the above study groups, with much higher frequencies than in milk from normal women of the general population in two different geographical areas studied (Philadelphia, Pa. and Washington, D. C.). Whereas the control studies in the latter areas showed positive specimens with frequencies of 4.5% (7/156) and 2.3% (1/43), respectively, the frequencies in the test-population groups ranged from 37.5% (6/16) for women who have had breast cancer, to 60% (6/10) for normal women belonging to high-breast-cancer families. The frequency for normal women of the Parsi sect was 39.1%, (18/46), and the average for all test groups was 41.7% (30/72). In addition to these highly significant results of studies involving milk, similar virus-like particles have also been observed in 11% (5/45) of tissue culture explants of breast cancer tissue maintained for 60 days or more, and in a continuous cell line from a pleural effusion associated with metastatic breast cancer.

As previously reported, candidate C-type viral agents have been isolated from a spontaneous breast cancer of a rhesus monkey and from a transplantable breast cancer of a laboratory rat. Both viruses are being successfully propagated in the laboratory, and are now being further investigated. Both have been shown to contain 70S RNA as well as the RNA-dependent DNA polymerase, characteristics of the RNA tumor viruses.

Segment Chairman. The program segment chairmen are concerned with the planning of the scientific projects to be accomplished within each working segment during the oncoming year. This critical function is performed on the basis of an analysis of the results of ongoing efforts, not only within the respective areas but also the efforts of all other program areas, as those efforts may pertain to the particular work of a program segment. It is on the basis of these overall plans that more specific work statements of the research to be performed are prepared and upon which contractors are invited to submit specific proposals for the performance of the work. By an integration of the research activity of these contractors, the segment chairmen are able to work toward fulfillment of the objectives of their segment.

In his capacity as chairman of the working group and in addition to the usual responsibilities associated with the effective functioning of advisory groups, the segment chairman is also responsible for bringing to the working group sessions all information relevant to the deliberations and decisions of the working groups, not only from the ongoing activities of the SVCP, but also from any other program areas of the Institute which may be relevant.

The program chairmen's meetings represent the major vehicle through which the chairmen execute their responsibilities as the focal point of total program operations and for which they are directly responsible to the Associate Scientific Director for Viral Oncology, the Scientific Director for Etiology and the Director of the Institute. The main function of these joint sessions is to provide the basis whereby a synthesis and integration of the activities of each segment is achieved with respect to the differential impact of these achievements on total program objectives. It is at the joint sessions that the relative priorities of research, which are established in each working area,are reviewed with respect to establishing an overall priority for the total program. This is an extremely difficult but important step because the items considered to be of high priority within the perspective of an individual program segment do not necessarily achieve the same level of priority within the perspective of total program. Thus, in joint session, simultaneously or in sequence, the chairmen perform the reviews, analyses, integrations and program decisions required for the effective attainment of the objectives of the total program.

Project Officers. Project officers are the direct extensions of the program segment chairmen regarding each particular contract within the segment. They carry to the contractor the reflection of the intent and philosophy of the objectives of the segment so that the contractors' performance contribute to the total goals and objectives of the segment and, therefore, the total SVCP. In order to do this, the project officer must have the scientific expertise necessary to act as an advisor to assure the accomplishment of the contract workscope with efficiency and effectiveness. This activity requires close and frequent communication between the project officer and the contract's principal investigator. The fine balance of coordination is maintained by frequent telephone conversations, written communications and by frequent visits between the project officer and the contractor. The functions of a good project officer require a lot of time which he might personally prefer to spend conducting his own research. The need, however, for existing and additional scientific personnel to continue and assume this managerial role cannot be overemphasized.

Executive Secretaries. The executive secretaries assist the segment chairmen and the project officers in the managerial duties that necessarily accompany the administration of a large research contract program. Each executive secretary is a senior scientist in his own field of specialty and thus consults with the segment chairmen, the project officers and the principal investigators representing the contractor in scientific aspects of the research being performed. A high level of scientific knowledge is necessarily required, since it is often impossible to separate scientific decisions from management decisions. Each executive secretary is specifically responsible for assuring that (1) each contract in his area receives optimal review; (2) working group members, as well as NCI and NIH officials are provided with required documentation; (3) appropriate recording of the proceedings of each meeting is made and that the information gleaned from the meetings is distributed to the proper scientific and management recipients; and (4) site visits are planned, implemented and documented as they are needed and required.

Contract Specialists. The contract specialists are the bridge between the scientists who administer research and the administrative mechanism whereby the research is implemented through the awarding and fiscal monitoring of a research contract. Within the Etiology Area, contract specialists are well conversant with scientific aspects of the program. They constantly provide invaluable advice in fiscal and legal matters to the project officers, the executive secretaries, the program segment chairmen and the Associate Scientific Director. It is the contract specialists, by combining their scientific comprehension with their administrative knowledge and skill, that allow the senior scientists to manage such a large and multidisciplined research program. This is an especially pertinent contribution to the total effort in the face of the present critical personnel shortages under which the program must and will continue.

Working Groups. The program segment working groups are the basic operational units for the SVCP. They are composed of both Federal and non-Federal scientists representing expertise in the varying areas of research involved in the program. Each working group is chaired by a senior NCI scientist. Although the primary function of the working groups is to provide advice to the Institute on the research and scientific aspects of the program, the participation of the members of the working groups is more extensive than the typical advisory committee. The members help and advise the respective chairmen in the development of program plans for their specific area of research for each fiscal year; develop work statements for specific research contracts; make site visits to contractor facilities; review contractor proposals; and when competitive proposals are received, select the best qualified contractor for recommendation to the Associate Scientific Director and participate in the very important functions of analyses and evaluation of the results of ongoing research accomplished in each of the segment areas. These analyses and evaluations provide basic recommendations for many major program decisions.

In addition to its advisory role to the segment chairman, the working group acts as a technical review group for the contracts within the segment. In this capacity, the working group acts as an advisory committee. It is asked to review individual contracts within the segment for scientific excellence and technical competence within the funding level, which is determined by the program and recommended for each individual contract. The final determination of the level of funding is dependent on many decisions which are expressed at the level of the Associate Scientific Director and the Scientific Director. This technical review group is asked to review contracts to determine (1) the appropriateness of the objectives of the contract as they fit into the overall goals of the segment's mission and in relation to other ongoing projects within the segment which might be related to the contract under scrutiny, and (2) the attainment of these goals, utilizing the scientific knowledge and methods available, to assure the highest technical performance of the contractor within the funding level allocated. Recommendations regarding funding are welcomed and seriously evaluated. However, the final decision will be made with consideration for the many variables, by the Scientific Director and the Associate Scientific Director for Viral Oncology, through their recommendation to the Director, National Cancer Institute.

Program Segment and Contract Reviews

Program Review. The contents of the total program are constantly being reviewed, both informally and formally, at various levels. The project officer, by his close contact with the contract's principal investigator, provides a continuous appraisal of each contract (1) for fidelity of performance of the contract (2) for assurance of enough flexibility in the contract to allow for changing and relevant program needs and (3) to assure that work under the contract is performed in the best technical way possible. Thus, the level of performance of each contract is constantly being considered by the segment chairmen through near day-to-day contact of each segment chairman with his project officers.

Each project officer must present and defend his contracts to the working group at the time of technical review. At this review, all aspects of the contract are considered in relation to the total segment's activities and responsibilities. At least once a year, each contract is presented by the program segment chairman to the Program Chairmen's review group where it is evaluated for relevance, priority and need to attainment of SVCP objectives and goals.

The SVCP is continuously changed, refined, reviewed, and evaluated by the Science Management Team and its key scientific advisors.

In summary, program review is effected at three levels, all of which are highly integrated and interrelated:

(1) The Contract is the basic functional unit and receives almost constant monitoring by the project officer and technical review by the working group who makes recommendations regarding goals and objectives to the Segment Chairman.

(2) Program segment working groups, as the basic operational units, review the contract performance for technical and scientific competence and adherence to contract workscope, for its contribution to segment goals and makes recommendations to the respective segment chairman concerning each of the segments activities.

(3) Program segment chairmen meeting jointly once a month, comprise the basic integrative unit and determine segment priorities within overall total program objectives and goals.

Contract Review. Contracts are reviewed according to their type.

Type I. The majority of the contracts in SVCP are of this type. These are research and development contracts which are generally limited to one or two areas of research and may include a service aspect. The dollar range is usually between $50,000 and $500,000 per year.

Type II. These are large and/or multi-faceted contracts which involve a number of research areas or have other special features. These are generally $1,000,000 and over and require special review consideration.

Type III. These are routine procurement contracts which cover the purchase of services and/or materials and contain essentially no research.

There are three major review groups: the Segment Working Group, the Program Segment Chairmen and the Etiology Program Management Group.

Progress Reports. As a valuable aid, in the review process, to all key members of the staff, the Program Analysis and Communications Section coordinates and handles progress reports on all SVCP contracts. Policy regarding progress reports was set forth in a memorandum dated November 6, 1968, from the Associate Scientific Director to the Segment Chairmen as follows:

"At a meeting of the Branch and Associate Branch Chiefs, Viral Oncology, on October 2, 1968, modified procedures for the preparation and dissemination of triannual project summaries from SVCP Segment Chairmen were discussed. In general, it was felt that the relatively small value of comprehensive overall program reports to contractors and working group members (on a triannual basis) was nearly completely negated by the time required for compilation, synthesis, and distribution of such reports. It was also felt that other more effective means of communication were already being utilized, and that such means which required less time of the program leaders should be strengthened and followed. Accordingly, the following recommendations were made and agreed upon for immediate implementation:

(1) Most contractors will continue to be required to submit short, concise, triannual progress reports in addition to their more comprehensive annual reports. As in the past, the latter is to be submitted in compliance with the directions of the Project Officer at the time the contract is considered for renewal.

(2) Management of triannual progress reports proceed as follows:

(a) Reports are received from all contractors by PAC between the 1st to 15th of October, February and June.

(b) Copies of reports are sent to the appropriate Segment Chairman by the 15th of these months.

(c) Each Segment Chairman with his Project Officers prepares one or several short paragraphs on highlights of progress. Each abstract is identified by contract no., title, and name of principal investigator. Abstracts of all progress reports are returned to PAC no later than the 30th of October, February 29th, and June 30th.

(d) Copies of all abstracts are duplicated by PAC and sent to all persons identified on the SVCP Route Slip.

(3) A single overall analytic report on progress, problems and projections of the SVCP is prepared on an annual basis by the ASDVO and distributed to all program personnel."

PROGRAM MANAGEMENT PERSONNEL

Science Management Team

Dr. J. B. Moloney, Associate Scientific Director for Viral Oncology, NCI
Mr. L. R. Carrese, Deputy Associate Director for Program, NCI
Dr. L. R. Sibal, Associate for Program Coordination, Viral Oncology, NCI
Dr. D. J. Rubin, Scientific Coordinator for Extramural Research, VO, NCI

Administrative Officer, Assistant Administrative Officers and Contract Specialists

Mr. John P. Patterson
- Mr. Nick Olimpio
- Mr. Robert Velthuis
- Mr. J. Thomas Lewin
- Mr. Maurice Fortin
- Mr. Thomas Porter
- Mr. Fred Shaw
- Mr. John Gibbons

Program Segments and Membership

Developmental Research Segment

Dr. Robert Manaker, Chairman
Dr. Jack Gruber, Vice Chairman and Executive Secretary
- Dr. Samuel Dales, Public Health Res Inst., N.Y.C.
- Dr. Paul Gerber, DBS, NIH
- Dr. Anthony Girardi, Wistar Institute
- Dr. Timothy O'Connor, NCI
- Dr. Alan Rabson, NCI
- Dr. Bernard Roizman, University of Chicago
- Dr. Duard Walker, University of Wisconsin
- Dr. Virginia Dunkel, NCI

Special Animal Leukemia Ecology Segment

Dr. Michael Chirigos, Chairman
Dr. John Glynn, Vice Chairman
Dr. George Burton, Executive Secretary
- Dr. Friedrich Deinhardt, Presbyterian St. Luke's Hospital
- Dr. Bernice Eddy, DBS, NIH
- Dr. Charles Rickard, Cornell University
- Dr. William Moloney, Peter Bent Brigham Hospital
- Dr. Mearl Stanton, NCI
- Dr. Peter Vogt, University of Washington
- Dr. David Yohn, Ohio State University
- Dr. Hans Sjogren, Pacific Northwest Research Foundation

Solid Tumor Virus Segment

Dr. Robert J. Huebner, Chairman
Dr. James Duff, Vice Chairman
Mrs. Harriet Striecher, Executive Secretary
- Dr. Charles Boone, NCI
- Dr. Maurice Green, St. Louis University
- Dr. Leonard Hayflick, Stanford University
- Dr. Karl Hellstrom, University of Washington
- Dr. Edwin Lennette, Calif. Dept. of Public Health
- Dr. Joseph Melnick, Baylor University
- Dr. Herbert Rapp, NCI
- Dr. Wallace Rowe, NIAID, NIH
- Dr. Hans Meier, Jackson Laboratories

Immunology Group

Dr. Paul Levine
Dr. Herbert Rapp Co-Chairmen
Dr. Ernest Plata, Executive Secretary
- Dr. Charles Boone, NCI
- Dr. Berton Zbar, NCI
- Dr. Tibor Borsos, NCI
- Dr. Ronald Herberman, NCI
- Dr. Maurice Hilleman, Merck Institute
- Dr. Barry Bloom, Einstein College of Medicine
- Dr. Arthur Brown, University of Tennessee
- Dr. George Santos, Johns Hopkins University

Resources and Logistics Segment

Dr. Robert Holdenried, Chairman
Dr. George Todaro, Vice Chairman
Dr. Roy Kinard, Executive Secretary
- Dr. James Duff, NCI
- Dr. K. Arnold Fowler, NCI
- Dr. Adi Gazdar, NCI
- Dr. Paul Levine, NCI
- Miss Marie Purdy, NCI

Biohazards Control and Containment Segment

Dr. Alfred Hellman, Chairman
Mr. Emmitt Barkley, Vice Chairman
- Mr. Mark Chatigny, Naval Biological Laboratory
- Dr. Peter Gerone, Fort Detrick
- Dr. Seymour Kalter, Southwest Foundation
- Dr. Maurice Mufson, Hektoen Institute for Medical Research
- Dr. William Payne, Div. of Environmental Health Sciences, NIH
- Dr. Briggs Phillips, Becton-Dickinson
- Dr. Arnold Wedum, Fort Detrick
- Dr. Richard Griesemer, Ohio State University
- Dr. Simon Sulkin, U. of Texas Southwestern Medical School
- Dr. George Michaelsen, University of Minnesota

Human Leukemia Therapy Segment

Dr. Seymour Perry, Chairman
Dr. Edward Henderson, Vice Chairman

Breast Cancer Virus Studies Segment

Dr. W. Ray Bryan, Chairman
Dr. Robert Depue, Vice Chairman
Dr. H. J. Clausen, Executive Secretary
- Dr. Louis R. Sibal, NCI
- Dr. Ernest Plata, NCI
- Dr. Harish Chopra, NCI
- Dr. J. R. Fraumeni, NCI
- Dr. Richard Bates, NCI
- Dr. E. Vollmer, NCI
- Dr. M. Black, New York Medical College, N.Y.C.
- Dr. M. Brennan, Michigan Cancer Foundation
- Dr. K. DeOme, University of California, Berkeley
- Dr. W. Feller, Georgetown University
- Dr. R. Gilden, Flow Laboratories
- Dr. D. Moore, Institute for Medical Research

Program Management Segment

Dr. J. B. Moloney, Chairman
Dr. L. R. Sibal, Executive Secretary
- Dr. W. Ray Bryan, NCI
- Dr. A. J. Dalton, NCI
- Dr. James Duff, NCI
- Dr. Michael Chirigos, NCI
- Dr. Robert Huebner, NCI
- Dr. Robert Manaker, NCI
- Dr. Deward Waggoner, NCI
- Dr. Robert Holdenried, NCI
- Dr. George Todaro, NCI

C. Scientific Activities - Progress Highlights:

There are now over 100 viruses which are known to cause virtually all kinds of cancer in every major group of animals including non-human primates. The probability is very high that viruses responsible for at least some human cancers will be found. When such agents are identified and successfully grown in the laboratory, it should be possible to develop therapeutic and preventive measures for the control of these diseases.

Viruses of one type (Type C) are known to cause leukemias, lymphomas, and sarcomas in chickens, mice, and cats. Particles, which closely resemble Type C viruses, can be found in human patients affected with these same kinds of cancers.

A monolayer culture releasing C-type particles has been established from cells of a pleural effusion of a child with Burkitt's lymphoma of the American type. The line is in its 28th subculture and continues to release virus. Studies have failed to identify this agent with known animal tumor viruses. This is the first human cell culture producing C-type virus in relative quantity. This makes it possible to produce antisera specific to a human C-type virus to be used for determination of the distribution of human C-type virus antigens in tumors.

Viruses of a second type (Type B) produce cancers of the breast tissue of mice. Thus far, this is the only animal species in which breast carcinoma has been proven to be caused by a virus. Recently, Type B particles resembling the mouse agent, have been found in human breast cancers and in milk concentrates from patients with breast cancer. Attempts to grow these viruses, if indeed they are definitely shown to be viruses, in the laboratory have just begun. Viruses of a third type (Herpes-type) which are structurally similar but unrelated to other known herpesviruses, have been shown to be associated with the following human malignancies: Burkitt's lymphoma, nasopharyngeal carcinoma, and uterine and penile carcinomas.

Hamster cells transformed by UV-irradiated herpesvirus type 2 grew as tumors when inoculated into newborn hamsters. This suggests that at least one virus which ordinarily is cytolytic may be oncogenic when an induced genetic defect blocking normal cycle of viral maturation with cytolysis results in the retention of the virogene in the replicating host cell.

By using techniques developed from animal tumor virus studies, every effort is being made to determine whether these viruses cause human malignancies. During the course of this work, it should be recognized that information on the biological, biophysical, and biochemical characteristics of cancer-causing viruses has provided a broad base of knowledge applicable to the isolation and identification of human agents and prevention and control of the disease in man. Some important new discoveries that have been made are: (1) Certain oncogenic viruses are unable to produce malignancies unless a "helper" virus is present, thus suggesting that an interplay exists between two viruses. (2) Certain tumor-inducing chemicals, irradiation, (carcinogens) may act as co-factors in activating latent, oncogenic viruses within cells. (3) Certain tumor viruses contain unique enzymes which are required in the replication of viruses in cells. These and other important developments will be discussed further in this report.

Type C Particles

When the Special Virus Cancer Program was initiated, highest priority was given to the search for human leukemia viruses resembling the Type C viruses causing chicken and mouse leukemias. Since that time, many Type C viruses, the total is now about 85, have been found in a variety of tumors from many species of two vertebrate classes. All of these species continue to be studied intensively under the broader scope of the Special Virus Cancer Program. Several of the Type C viruses are established as the causative agents in leukemias, lymphomas, and sarcomas of chickens, mice, cats and hamsters. Many of these can infect and produce malignancies in other species (e.g. a sarcoma virus of the cat produces tumors in marmoset monkeys). Furthermore, some of these viruses can cause malignant transformation to occur in animal and human cells grown in the laboratory (e.g. cat leukemia and sarcoma viruses alter embryonic human cells). Type C virus particles have been found in association with malignancies of a spectrum of animal species including non-human primates, rats, cattle, woolley monkeys, gibbons, and man. Although electron microscopy is an important method in the search for Type C virus particles, certain newer biochemical, biophysical and immunological techniques, developed through intensive collaborative studies of animal cancer viruses, are now being directed toward finding viruses present overtly or covertly in the cells of human cancer.

1. Reactions between Type C viruses causing leukemias and sarcomas (solid tumors)

When inoculated into appropriate cell cultures, Type C sarcoma viruses of chickens, mice and cats produce foci of altered cells. This fundamental discovery provides a readily visible indicator reaction for the detection of sarcoma viruses. On the other hand, leukemia viruses grown in tissue culture do not cause foci or other detectable changes. The finding that leukemia viruses can either inhibit or enhance focus formation by sarcoma viruses of the same species has led to the development of methods for the detection and quantitation of leukemia viruses indirectly.

Certain of the chicken, cat and mouse sarcoma viruses are "defective" in that they do not produce foci in cell cultures or tumors in animals in the absence of a co-infecting, "helper" leukemia virus. Further, in the presence of a defective sarcoma virus the helper action of leukemia viruses can be used as a specific indicator for their detection and quantitation. It is now believed that defective sarcoma virus--leukemia virus interactions may be more widespread in nature than originally thought and that similar systems may be found in man. A mouse leukemia virus which has been adapted to grow in human cells is now available to search for defective human sarcoma viruses, if they exist. An alternative approach has been developed for the detection of possible human leukemia viruses. A defective mouse sarcoma virus and its leukemia virus helper can be made to form tight functional aggregates, which behave as one virus. Using a mixture of a mouse sarcoma virus and a cat leukemia virus, a hybrid aggregate which could be grown continuously in cat cells was produced. Because the aggregate is defective, it requires the simultaneous presence of a cat leukemia virus for producing altered foci in cat cells. Thus a focus forming sarcoma virus of the mouse, artificially changed to one possessing infectivity for cat cells, can now be used in cultures for the detection of cat leukemia viruses.

This hybrid virus, as well as the cat leukemia virus, will also grow in human embryonic cells in tissue culture. If sufficient amounts of the Type C particles found in association with human leukemia can be obtained, the possibility exists that the cat-adapted mouse sarcoma virus can be hybridized with the human agent to produce an indicator system for the detection of human leukemia viruses.

2. Ubiquity of Type C viruses and their possible transmission in nature as part of the genetic inheritance.

By developing highly sensitive immunological methods for detecting Type C viruses of chickens, mice and cats, an extensive program to determine the occurrence of Type C viruses throughout the life span of different animals has begun. Studies in mice revealed that no strains are completely free of Type C virus involvement. In strains subject to a high incidence of spontaneous leukemia, the virus could be demonstrated even in fetal animals and at all ages after birth; in strains with a very low incidence of leukemia, the virus could not be detected at any age under natural conditions. If the latter mice were irradiated or treated with cancer-causing chemicals, Type C virus could be readily detected well in advance of the tumors that developed. Mice of strains with moderate incidences of leukemia showed no detectable virus during the first third of their life spans, but Type C virus appeared spontaneously and could be detected with increasing frequency as they became older and developed leukemia and other cancers. Irradiation or treatment with chemical carcinogens, however, resulted in detection of Type C virus early in life and in the development of cancers. These and other findings led the investigators to postulate that the genetic information of the Type C viruses is transmitted "vertically" (from parent to offspring) in a covert form along with the genes bearing the normal genetic information of the animal. Like normal genes they can be repressed and show no action under some circumstances, but can become derepressed under other circumstances, such as during aging or under the influence of irradiation or carcinogenic chemicals. This theory has been supported by the demonstration of "indicators" of the presence within tumor cells of viral material even in the absence of detectable whole virus particles. These "indicators" or group-specific antigens (gs antigens) are specific subunits of the internal structure of the Type C RNA tumor viruses. One type of group-specific antigen is common only to the viruses of a particular animal species (species specific) and has now been demonstrated in the embryonic tissues of most mammalian and avian species. This suggests the possible role of these viruses in normal cell growth and differentiation as well as in the induction of malignancy.

Very recently, a second group antigen associated with Type C viruses has been demonstrated. Preliminary evidence would indicate that it may be an interspecies-specific group antigen in that it is present and common among the Type C viruses of 3 mammalian species, the mouse, cat, and hamster, but not the chicken. Furthermore, this antigen has been detected in highly concentrated extracts of cells from two different human tumors, one of which has at times produced small amounts of a virus resembling animal Type C particles. If confirmed, the implication of this finding is obvious--a formidable probe for the presence of virus or viral genetic material in human cells has been developed.

Projecting that Type C viruses will be found to be as ubiquitous in man and other vertebrates as in mice, chickens, and the cat, the hypothesis is proposed as a unifying theory consistent with the phenomena of radiation and chemically induced cancer as well as the predictable occurrence of spontaneous cancers that develop in various populations of animals, including man. An exciting consequence of this theory, even if it is found to hold only for a limited number of cancer types in man, is the prediction that a new approach may become available for the control of these cancers, namely the delineation of factors responsible for both derepression and repression of virus expression.

A somewhat different approach has been based on the premise that tumor cells containing a common antigen may indicate a similar viral causation. In examining the tissues from various human sarcomas, particles resembling known animal Type C viruses were found in at least one liposarcoma. With continued culture of the tumor, particle production ceased, but immunologic tests using the serums of sarcoma patients revealed that the cells still contained a substance (an antigen) which appears to be present only in sarcoma cells (sarcoma-specific). A high incidence of serum antibody to sarcoma antigen was found in patients with various types of sarcomas and their close associates; a significantly lower incidence was found in normal serums. The data suggest that a viral agent may be the causative factor in this disease. Hopefully, continuing studies will establish a more definite relationship of the antigen to this disease.

3. New approaches to detection and control of Type C virus infections.

Increasing evidence has accumulated to substantiate that the genetic material of Type C RNA viruses can become integrated into the host-cell DNA (mammalian genetic material). A dramatic breakthrough in the investigation of the biochemical pathways of tumor virus infection and replication has been the demonstration that RNA viruses contain enzymes called polymerases which can direct the synthesis of DNA by the host cell. In rapid succession many investigators found that one of these enzymes (RNA-dependent DNA polymerase) is associated with all of the known RNA Type C tumor viruses. Viruses that cause leukemias or sarcomas in chickens, mice, cats and hamsters, as well as breast cancers in mice, rats, and monkeys, have been shown to contain polymerase activity. By contrast, well-characterized, non-tumor producing viruses do not contain this activity. This research has resulted in the development of new, extremely sensitive methods for the detection of RNA tumor viruses in human cancer. Assays for the polymerase enzymes exceed by 100-fold the sensitivity of electron microscopy and are capable of detecting viruses which are present either in latent form or as whole virus particles.

Intensive investigations have now revealed polymerase activity in cells of patients with acute lymphoblastic leukemia; more preliminary evidence has shown the enzyme is in cells of sarcomas, Burkitt's lymphoma and breast cancer. Since the RNA-dependent DNA polymerase is apparently always present in the RNA tumor viruses of animals, its discovery in the human tumor cells offers good supportive evidence that viruses are associated with cancers in man. The RNA-dependent DNA polymerase of human leukemia cells is inhibited by a drug, n-demethyl rifampicin, which also inhibits the enzyme activity found in the Type C RNA tumor viruses of animals. Studies are underway to explore the action of this drug and the various modifications of it. These investigations could provide new approaches to the treatment of malignancies in man.

Type B Particles

"Type B" viruses causing breast cancer in mice possess certain properties in common with Type C viruses, such as having genetic material of the RNA type and being transmitted from parent to offspring along with the normal genetic inheritance. But they also differ in: (1) the manner in which the nucleoid is formed during virus reproduction; (2) the fine details of their ultrastructural appearance; and (3) transmission in infectious form, under natural conditions.

The potential existence in humans of an infectious breast cancer virus similar to that of mice, together with epidemiological evidence of "clustering" of breast cancer in some human families similar to that observed in the earliest studies of cancer in mice, led to systematic viral studies on this human disease. Particles resembling the Type B virus of mouse breast cancer have been observed in 40% or more of milk specimens from women with breast cancer, as well as from healthy women of high-risk populations (high-breast-cancer-families, inbred Parsi sect of Bombay, India), as compared with a frequency of only about 6% for specimens from healthy women of the general population. Similar particles have also been observed in two tissue culture lines of human breast cancer that have been successfully grown in the laboratory. More recent studies of one of these cell lines have revealed additional evidence consistent with presence in the cells of an RNA tumor virus: the presence of the RNA-dependent DNA polymerase, the interspecies antigen.

Breast cancer occurs in about 4 to 5% of American women. It is the most prevalent and responsible for more deaths than any other type of cancer, not only in American women but also among women of several other countries. Because breast cancer occurs 2 or 3 times more frequently in some families than in others it is strikingly similar to observations made on breast cancer of different populations of mice. These animal studies led to unequivocal evidence of the association of a virus and the demonstration that an infectious form of it is transmitted through milk. One of the major objectives of the SVCP is now the determination whether an agent similar to that of mice is responsible for the unusually high incidence of breast cancer in certain human populations.

Herpes-type Viruses

A type of virus associated with some forms of chronic leukemia, lymphoma, and postnasal carcinoma is the herpes-type virus (HTV), similar in size and shape but not identical to other known herpesviruses. Unlike human Type C particles, HTV grows well in the laboratory and fairly large quantities of purified and concentrated HTV can be recovered for study. One of the most active areas of viral oncology is that concerned with definitive characterization of the HTV.

Considerable interest has developed in the herpes group of viruses as cancer-causing agents in animals and man. Herpes-type viruses have been shown to induce kidney carcinomas of the frog and to be causally related to lymphoproliferative diseases in chickens, monkeys, marmosets and rabbits. Projects within the Program have focused on the significance of the Epstein-Barr virus (EBV) from Burkitt's lymphoma and postnasal carcinoma and Herpesvirus hominus type 2 (HSV-2) from cervical carcinoma.

Seroepidemiological studies on the relationship of EBV infection to nasopharyngeal cancer are being conducted through the International Agency for Research on Cancer. A study in the West Nile District of Uganda to determine the feasibility of further studies on EBV in relation to Burkitt's tumor is nearing completion. Other studies also suggest an association between infection by HSV-2 and cervical carcinoma. Results of a study made in Texas showed the presence of serum antibodies to this virus in about 85% of cases of invasive cervical carcinoma in comparison to 22% in controls. Recent findings in Colombia showed a much higher incidence of antibody in the control population selected, approximating the incidence in the tumor-bearing group. At present, insufficient data is available to conclude that this virus is implicated in this cancer.

EBV infection has been associated with the development of infectious mononucleosis in young adults, a disease with the attributes of a self-limiting leukemia. The generally high levels of antibodies to EBV in patients with Burkitt's tumor or with nasopharyngeal carcinoma also suggest that the virus might be causally-related to these diseases; high levels of antibody also occur in patients with the sarcomatous form of Hodgkins disease, chronic lymphocytic leukemia, and sarcoidosis. Present methods show no significant differences between EBV isolated from these different diseases.

Several facts make EBV suspect as a prime if not a sole factor in the induction of hematological disease in man: (1) The virus is ubiquitous; no human population has been found to be free of antibody to EBV. (2) The virus is consistently found in association with Burkitt's lymphoma. (3) Only lymphoid cells appear to be susceptible to infection. (4) Lymphocytes from negative donors have not been capable of continuous cultivation unless they are infected by the virus. (5) Infected continuous cell lines which do not releave virus contain soluable antigens that react with serums containing antibodies to EBV. (6) EBV has been strongly associated with infectious mononucleosis, cell lines of which grew as tumors in immunosuppressed hamsters. (7) Other herpesvirus of animals have been causally related to lymphoproliferative diseases. (8) The genetic material of EBV is related to that of similar viruses known to induce malignancies in animals. Thus, EBV has several features characteristic of known tumor viruses. Current data suggest that: genetic material of EBV may be incorporated into lymphoid cells; mature virus production may be a rare event; infected cells acquire the capacity for unlimited growth and produce tumors in the immunologically deprived host; infection by the virus may be a factor in tumor development under certain unknown conditions affecting the immunity of the patients.

D. Projections:

Based largely on the recent findings from work conducted within the Special Virus Cancer Program, the following broad areas of research will be developed and/or expanded: (1) Virus (or viral antigens) - tumor relationships, (2) Molecular studies, (3) Immunologic studies, (4) Test systems, and (5) Resources.

1. Virus (or viral antigen) - tumor relationships

   a. Model Studies. Studies on animal, RNA and DNA, tumor viruses, known to cause malignancies in several mammalian species, will be continued. The results of these studies have already provided a broad base of knowledge on the characteristics of tumor viruses applicable to the isolation and identification of human agents. This work will remain an integral part of the Program.

   b. Human Studies. Efforts to identify viruses or detect virus expression in human tumors are underway. The Program is now prepared to broaden its activities to search for candidate viruses or subviral products which induce human malignancies as follows:

      (1) To identify and isolate candidate viruses or subviral products in leukemias, lymphomas and sarcomas.

      (2) To identify and isolate candidate viruses or subviral products in breast, lung and other carcinomas.

      (3) To develop methods for the detection of high cancer risk groups, i.e. individual susceptibility of predisposition to transformation by human viruses.

      (4) To extend present and develop new methods for the successful propagation of significant amounts of human candidate viruses.

      (5) To develop suitable reagents for mass diagnostic screening for candidate viruses.

      (6) To characterize, biologically and biochemically, presumptive viral agents.

      (7) To increase emphasis on understanding the relationship of environmental agents (e.g. chemical carcinogens) as co-factors in viral carcinogenesis. This represents a major expansion of effort requiring combined efforts of the Viral Oncology and Chemical Carcinogenesis Areas.

2. Molecular Studies

In recent months rapid major advances have been made in the field of molecular biology. These findings have direct application to the study of the relationship of viruses to tumors. There is evidence that the genetic material (RNA) of the tumor viruses can direct the synthesis of new DNA. The demonstration that RNA tumor viruses contain enzymes (polymerase, ligase) which may be required for viral infection, interaction with host cell genome, and viral replication has provided the basis for the development of new,extremely sensitive methods for the detection of oncogenic viruses or their "fingerprints." Indeed, knowledge of the fundamental molecular events which occur during virus infection and subsequent cell transformation provides the first truly rational approach to therapy. Enzyme activities analogous to those of RNA tumor viruses have recently been found in cells of human leukemics. This offers strong supportive evidence that viruses are associated with cancers in man.

a. Basic studies

The Program is prepared to broaden its activities for identifying and characterizing the spectrum of enzymes (and other mediators) required by tumor viruses for replication and transformation.

b. Applied studies

As knowledge of the fundamental molecular events in virus-cell interactions is developed, the Program will apply this information to the study of human cancer as follows:

(1) To identify and characterize similar enzymes or enzymatic activities within normal and malignant human cells.

(2) To develop highly sensitive methods for the detection of virus or virus activity in human cells.

(3) To develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral replication and/or tumorigenesis at the cellular and subcellular levels. The therapy could be directed at any or all of the stages of cell transformation beginning with cell infection by a tumor virus.

Ultimately these studies will require an exhaustive effort to develop drugs, anti-enzymes, gene repressors or inhibitors effective at the molecular level.

3. Immunological Studies

Immunologic research has provided extremely sensitive techniques for detection and characterization of tumor viruses, viral antigens, and changes in surface membranes of tumor cells. Indeed, such efforts have contributed to an understanding of the role of immunological mechanisms in host-tumor and host-virus interactions which provide an approach to the prevention and treatment of cancer.

a. Basic studies. Investigations of selected model systems, representing tumors induced by Type C, Type B, and Herpes-type viruses, will be extended to further identify, characterize and determine the viruses, viral antigens, and membrane antigens of tumor cells. This includes development and application of improved techniques with the sensitivity and specificity required to detect cellular alterations induced by tumor viruses alone or as the result of interaction with other environmental agents (e.g. chemicals, irradiation). Efforts will be increased to develop similar immunological methods and diagnostic reagents for application to human cancer. Research will be intensified and expanded:

   (1) To study cellular and humoral immune mechanisms and to determine their relative significance in host recognition of and response to tumor and/or tumor viruses.

   (2) To develop methods to enhance host response to tumor or virus antigens.

Increasing emphasis will be directed toward research on spontaneous or naturally occurring tumors in model systems relevant to human cancer. These studies would provide the basis for a rational approach to prevention (vaccines) and treatment (immunotherapy) of cancer.

b. Applied studies. Basic research will provide the framework for identification and characterization of viruses, viral antigens, and cell membrane alterations in human cancers. Immunological methods and reagents will be developed and applied:

(1) To relate candidate human viruses to known oncogenic agents.

(2) To identify and characterize interspecies viral antigens which are present in known mammalian tumors, and therefore, could provide the basis for a formidable probe to detect human tumor viruses or viral antigens.

(3) To launch large-scale seroepidemiological surveys which will define high risk populations.

(4) To determine the presence of cross-reacting antigens in various human tumors.

Clinical studies will be directed toward understanding and manipulation of immune mechanisms in human cancer as a basis for:

(1) Development of vaccines from identified and fully-characterized human tumor virus (es).

(2) Determination of the role of host immune responses in tumor recognition and rejection.

(3) Application of (1) and (2) in the prevention and control of human cancer.

As research progresses, increased emphasis on application will be as follows:

(1) Immunodiagnosis and seroepidemiology
(2) Clinical studies on the role of immune mechanisms in human cancer
(3) Immunotherapy
(4) Vaccines (conventional or other)

Ultimately, these studies would be organized to coordinate and integrate the application of appropriate biochemical, immunological, and genetic methods of detection, prevention, and control of various types of human cancer.

4. Test Systems

In vitro and in vivo (animal) test systems will be carefully selected to evaluate the work outlined in the previous research areas; specifically: (a) to determine the oncogenic potential of candidate human viruses; (b) to develop bicassay systems for testing viral, and viral/chemical carcinogens; (c) to begin vaccine (conventional or other) testing and immunization programs; (d) to begin therapy testing programs; and (e) to explore special animal tumor systems with particular relevance to human cancer.

Well-characterized cell culture lines and virus-defined animal stocks (small mammalian and primate species) will be developed and maintained.

5. Resources

Research efforts will undergo continual change in emphasis and scope as new leads emerge. A variety of resources will have to be developed, maintained, and coordinated within a flexible program which meets these needs. This requires: (a) Human Resources - collection and storage of serum and tissue specimens, integration of data on clinical records, storage and distribution; computerization, coordination of specimen collection, storage, and distribution; (b) Animal Models - maintenance of various mammalian animal colonies for basic research and special studies; (c) Reagent Production - large scale production of animal tumor viruses for basic research; production of standardized lots of purified viruses; and production of high quality diagnostic reagents; (d) Candidate Human Virus Production - intensive developmental research effort to isolate and produce human viruses; and (e) Biohazards Control and Containment - controlled environment facilities are required for research on known oncogenic viruses and candidate human tumor viruses as well as for maintaining animal colonies which are protected from extraneous infections. To fulfill this need, a substantial and costly effort is required to provide research, supportive services, and advice to the Program's research laboratories.

SPECIAL VIRUS CANCER PROGRAM

FUNDING LEVELS OF CONTRACTS WITHIN SEGMENTS
(By Type of Institution)
VIRAL ONCOLOGY AREA, ETIOLOGY, NCI

Segment	Profit		Educational		Non-Profit		Other*		Total	
	No.	Amount **	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Program Management	9	4,129	4	1,707	3	377	4	1,314	20	$7,527
Developmental Research	3	5,070	10	2,653	9	1,182	0	0	22	$8,906
Solid Tumor Virus	1	2,610	7	3,585	6	534	0	0	14	$6,729
Special Animal Leukemia	2	820	11	2,654	3	305	1	144	17	$3,924
Immunology	1	149	3	468	2	327	0	0	6	$944
Resources & Logistics	5	1,155	10	722	5	133	0	0	20	$2,011
Breast Cancer Virus Studies	1	100	1	172	2	434	0	0	4	$706
Biohazards & Environ. Cont.	2	424	2	174	-	---	1	33	5	$631
Total	24	14,458	48	12,137	30	3,293	6	1,491	108	$31,379

* Includes interagency agreements
** Dollars in Thousands

SPECIAL VIRUS CANCER PROGRAM

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SUMMARY REPORT

Office of Biohazards and Environmental Control

The Office of Biohazards and Environmental Control conducts research pertaining to the physiological and environmental factors that alter host susceptibility and response to oncogenic and non-oncogenic viral infections. It also evaluates and develops techniques and equipment to minimize cross-infection and provide adequate environmental controls.

The Biohazards Section conducts research in an effort (1) to describe the role of immunocompetence in oncogenesis; (2) to define biochemical factors which might lead to the induction of malignancy and how best to detect and modify these inductive changes; and (3) to develop *in vivo* and *in vitro* systems to assess the host response to physiological imbalance.

A sensitive and reliable system to culture mouse lymphocytes, responsive to specific and non-specific antigenic stimulation, has been developed. Hormones associated with normal physiological functions have been shown to alter blastogenesis. It has further been established that several chemical and physical factors are required in the process of blastogenesis. Using Type C RNA viruses and myxo-viruses we have also demonstrated blastogenic repression associated with oncogenic virus infection in contrast to blastogenic enhancement with myxo-virus infection. We have further demonstrated that many hormones influence the derepression and possibly the repression of group-specific-antigens of the Type C RNA viruses.

Alteration of certain species of tRNA have been suggested during early stages of oncogenic virus infection. Thus far, we have observed at least one altered species of tRNA associated with tumor virus-infected mouse tissue.

The Environmental Control Section uses environmental monitoring as a method for identifying sources of contamination. To assure the integrity of primary and secondary biological barrier systems, they evaluate engineering and operational parameters and their effect on experimental and personnel contamination control. Similarly, they collect and evaluate information pertinent to biological safety and evaluate methods for handling potentially hazardous agents. This approach has resulted in improved performance of biological barriers and has served as a basis for developing more efficient and safer operating procedures. Thus, more flexible and safer laminar flow cabinetry is currently under fabrication and will undergo in-use testing. Physical tracer systems are being developed to aid in the evaluation of dynamic containment equipment to assure proper use of facilities and to maintain a high level of laboratory safety practice.

Serial No. NCI - 4805
1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical Parameters Immunosuppression in the Malignant State

Previous Serial Number: Same

Principal Investigator: Dr. Alfred Hellman

Other Investigators: Dr. Arnold K. Fowler
Dr. Harry G. Steinman

Cooperating Units: None

Man Years:

Total	2.5
Professional:	0.5
Others:	2.0

Project Description

Objectives:

A. The development of an understanding of biochemical factors that lead to the induction of malignancy and to determine how rapidly and efficiently these inductive changes can be detected and modified.

B. Evaluation of host factors that influence malignancy.

Methods Employed:

A. The influence of viral infection on early responses of the host as demonstrated by changes in protein synthesis has recently come under intensive investigation. The primary system currently being investigated is the interaction of bacteriaphage and its host system. In this type of study change in species of tRNA are identified after less than two minutes of viral infection. Utilizing these techniques the influence of host environment and viral induced tumor formation is being investigated.

B. Cellular immuno-competence such as hypersensitivity, lymphocyte transformation as well as other indications of immuno suppression are being investigated.

Major Findings:

To date we have been investigating the influence of *in vivo* and *in vitro* environment on the specificity of isoaccepting transfer RNA of L-M cells. In an associated *in vivo* study we have been able to demonstrate the influence of the endocrine environment on tumor expression and derepression.

A. We are currently looking at the tRNA species of normal BALB/c mice and RLV infected BALB/c mouse spleens. Thus far we have been able to observe at least one altered species of tRNA in the infected spleen. We are continuing to look for further evidence of alteration in biochemical parameters during early stages of oncogenic viral infection.

B. We are also determining the influence of co-infection of tumor induction as well as its influence on the cellular immune mechanisms. Utilizing RLV and PR-8 infection we have been able to demonstrate lymphocyte depression associated with RLV and enhancement with PR-8. We are pursuing ramifications relating to the induction of murine leukemia and the general host response to murine oncogenic viruses.

Significance to Biomedical Research and the Program of the Institute:

The elucidation of the molecular events leading to malignancy as well as its implication in immuno-competence will provide a more rational means for control and therapy. An understanding will provide a more meaningful approach to studying factors involved in tumor induction by biological and physical means. These studies should also lead to a more rapid means of detecting the transforming or malignant state in the host.

Proposed Course:

The development of *in vitro* assay system for studying early inductive changes during viral produced malignancy and to determine the genetic and immunologic factors associated with such inductions and its modification.

Honors and Awards:

A. Co-Chairman of the Virology Session, Tissue Culture Association Meeting, June 7, 1971, Lake Placid, New York.

B. Presented invited lecture of the Bar Harbor Seminar Course on Virology, July 20, 1970. The lecture was entitled: Current Trends in the Efficient Utilization of a Virology Laboratory.

Publications:

Hellman, A., Fowler, A. K., Steinman, H. G., and Bovich, P. M.: Lymphocyte transformation with oncogenic and non-oncogenic murine virus. In Vitro (1971)

Hellman, A., and Fowler, A. K.: Activation of group specific C-type RNA tumor virus antigens by steroid hormones. Bact. Proc. (1971)

Fowler, A. K., Hellman, A., Steinman, H. G., and Quatrale, A. C.: The effect of progesterone on mouse lymphocyte transformation. In Vitro (1971)

Hellman, A. and Fowler, A. K.: Hormonal activated expression of the C-type RNA tumor virus genome. Nature (in press)

Fowler, A. K., Hellman, A., Steinman, H. G., and Quatrale, A. C.: Studies on the blastogenic response of murine lymphocytes. I. Quantitative measurements of stimulation by phytohemagglutinin. Proc. Soc. Exp. Biol. Med. (in press)

Serial No. NCI - 4806
1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Environmental Control Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of Biological Safety and Environmental Control Equipment, Facilities and Procedures

Previous Serial Number: Same

Principal Investigator: Mr. W. Emmett Barkley

Other Investigators: Dr. Alfred Hellman

Cooperating Units: OUTSIDE NIH
Industrial Hygiene and Safety Directorate
Ft. Detrick, U. S. Army

Man Years

Total	2.0
Professional:	2.0
Other:	None

Project Description

Objectives:

The primary objectives of the biological safety and environmental control evaluation project are: (1) to establish an environmental monitoring program for the identification and resolution of biocontamination problems and to provide continuous surveillance of the primary and secondary biological barrier systems which have been incorporated into the National Cancer Institute's virus containment facility; (2) to evaluate engineering and operational parameters of prototype virus-cell culture laminar flow safety cabinets and to determine the effects of those parameters on experimental and personnel contamination control; (3) collect and evaluate information pertinent to the biological safety aspects of procedures, equipment and facilities; (4) to develop a current inventory of agents studied in research laboratories supported by the Special Virus Cancer Program; and (5) to evaluate methods for handling potentially hazardous materials and infected animals.

Methods Employed:

Standard engineering methods for analysis of air flow characteristics and pressure differentials are employed. Physical and biological tracers are employed for determining the capability of facilities, equipment and procedures to control biological hazards under a variety of mechanical and operational stress situations. Volumetric air samples and airborne particulates are collected by standard environmental monitoring and industrial hygiene techniques.

Site visits to various virus laboratories associated with the Special Virus Cancer Program, using appropriate evaluation forms, are employed to observe the performance of safety procedures, equipment and facilities. Environmental monitoring surveys are performed to determine the degree and source of intramural biocontamination and to identify all factors which may create unsafe conditions. Data from the site visits and environmental monitoring surveys are evaluated to determine the capability of the laboratory to perform potentially hazardous research.

Major Findings:

An environmental monitoring program is a valid method for identifying sources of contamination and for assuring the integrity of primary and secondary biological barrier systems. Information from this program has resulted in improving the performance of biological barrier systems and has served as the basis for developing safer and more efficient operating procedures. For example, the environmental monitoring program associated an increase in intramural contamination with improper laundry handling procedures. These procedures were changed to alleviate this contamination problem.

The preliminary site visits to three contract facilities have resulted in the identification of numerous facility and equipment deficiencies and inadequate laboratory practices. Improved procedures have been recommended and are being implemented. It has been determined that approximately 95 percent of all laminar flow safety equipment used by these contractors does not meet the minimum safety specifications.

An experimental laminar flow safety cabinet has been designed to provide the capability for changing cabinet dimensional parameters and air flow parameters. This cabinet can provide controlled air turbulence zones for various air supply and exhaust conditions. Air velocity profiles and filter efficiencies can be maintained at required performance levels. This demonstrates that the operation of the laminar flow safety cabinets under controlled conditions has minimal effect on the air velocity and filter efficiency parameters.

Significance to Biomedical Research and the Program of the Institute

This project will improve the quality and safety of research laboratories associated with the National Cancer Institute's Special Virus Cancer Program. Results from these studies will serve as a basis for establishing standard operating procedures for virus laboratories and appropriate design criteria for containment equipment. Currently there is a paucity of biological safety information pertinent to virus research. This project will contribute to alleviating this deficiency by disseminating practical biological safety information.

Proposed Course

The environmental monitoring study will be continued to further understand the capabilities of primary and secondary biological barrier systems to protect the environment and personnel from exposure to potential biological hazards and to protect the experiment from contamination.

A physical tracer system utilizing a carrier gas and entrained fluorescent particles will be developed to evaluate the containment capability of laminar flow safety cabinets. This technique will also be used to evaluate the effect of personnel activity on the containment performance of four standard laminar flow safety hoods.

In addition, innovative approaches to the design of safety equipment will be developed to improve the capability for virus containment without concomitant personnel constraints.

Biological safety and environmental control evaluation surveys will be performed at all major SVCP contract facilities. Follow-up consultation activities will be initiated to assist the SVCP contractors in implementing recommendations to improve laboratory safety.

Honors and Awards:

1. Mr. Barkley was invited to present a paper entitled "A New Era of Biological Safety Cabinets" at the Tenth International Congress of Microbiology, which was held in Mexico City August 9-15, 1970.

2. Mr. Barkley has been invited to present a paper entitled "Training in Use of Safety Devices, Including Laminar Flow Equipment" at the 14th Biological Safety Conference, April 13-15, 1971 at the Center for Disease Control, Atlanta, Georgia.

3. Mr David West has been invited to present a paper entitled, "Maintaining Correct Airflow in a Containment Laboratory" at the 14th Biological Safety Conference, April 13-15, 1971 at the Center for Disease Control, Atlanta, Georgia.

4. Mr. Barkley has been invited to be a principal speaker at the Research and Development Symposium sponsored by the National Safety Council at the University of Minnesota, Minneapolis, Minnesota, June 14 and 15, 1971. This presentation will be entitled, "Safeguards in Microbiological Laboratories".

Publications:

None

Serial No. NCI - 4807
1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunocompetence and Susceptibility of Animal Systems to Oncogenesis

Previous Serial Number: Same

Principal Investigator: Dr. Arnold K. Fowler

Other Investigators: Dr. Alfred Hellman
Dr. Harry G. Steinman

Cooperating Units: None

Man Years:

Total	2.1
Professional:	1.1
Others:	1.0

Project Description

Objectives:

To elucidate the biological mechanisms underlying host immunocompetence and ascertain their involvement in oncogenesis. Physiological imbalances, induced by controlled stresses, will be examined at the biochemical level as causative factors in host immunological inadequacy. In vivo and in vitro model systems will be developed to assess and monitor host immunocompetency.

Methods Employed:

The in vitro blastogenic response of lymphocytes to specific and non-specific antigen stimulation is being examined for its potential value as an index of host immune status with the ultimate intent to study mechanisms of physiological actions on lymphocyte responsiveness to infectious challenge. This is being supplemented by conventional serological methods to detect group specific antigens of the C-type RNA virus and certain biochemical assay techniques to determine enzyme levels in rapidly proliferating tissues. Many of these studies have also been supported by electron microscopy.

Major Findings:

A sensitive and reliable system to culture and study the blastogenic response of splenic and peripheral mouse lymphocytes to specific and non-specific antigenic challenge has been described. Using this system, various endocrines (steroids) associated with normal physiological functions have been shown to alter lymphocyte blastogenesis. Similarly, we have demonstrated that many of these endocrines also influence the de-repression and possibly the repression of the group specific antigen of the C-type virus.

Significance to Biomedical Research and the Program of the Institute:

The essential role of the host immune response in the etiology of some, if not all cancers, has become increasingly apparent during recent years. The concept that degradation of natural immunity processes under certain environmental and/or physiological stress precede or are concomitant with infection by oncogenic viral agents is not new, although it remains poorly defined. The frequent implication of host physiology in the development and pathogenesis of cancer, as well as their involvement in host immunocompetence, points to the need of further research directed toward elucidating the biochemical mechanisms and the degree of its participation in these phenomena as a prerequisite to the development of means to control human cancers.

Proposed Course:

The use of *in vivo* and *in vitro* model systems will be employed to examine the involvement of host physiological-immunological interactions in the induction and propagation of the malignant state. Concentrated efforts will be continued to develop new and improved criteria to study these interrelationships.

Honors and Awards:

None

Publications:

Fowler, A. K., Hellman, A., Steinman, H. G., and Quatrale, A. C.: The effect of progesterone on mouse lymphocyte transformation. *In Vitro* (1971)

Hellman, A., Fowler, A. K., Steinman, H. G., and Bovich, P. M.: Lymphocyte transformation with oncogenic and non-oncogenic murine virus. *In Vitro* (1971)

Hellman, A., and Fowler, A. K.: Activation of group specific C-type RNA tumor virus antigens by steroid hormones. *Bact. Proc.* (1971)

Bardell, D. and Fowler, A. K.: Inhibition of dehydrogenase activity in lung tissue due to breathing oxygen at atmospheric pressure. Aerospace Medicine (in press)

Hellman, A. and Fowler, A. K.: Hormonal activated expression of the C-type RNA tumor virus genome. Nature (in press)

Fowler, A. K., Hellman, A., Steinman, H. G., and Quatrale, A. C.: Studies on the blastogenic response of murine lymphocytes. I. Quantitative measurements of stimulation by phytohemagglutinin. Proc. Soc. Exp. Biol. Med. (in press)

Serial No. NCI - 4808
1. Office of the Associate Scientific Director, Viral Oncology, Etiology
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the Cellular Immune Defense Mechanisms in Response to Tumor Induction

Previous Serial Number: Same

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. Arnold K. Fowler
Dr. Alfred Hellman

Cooperating Units: None

Man Years:

Total	1.5
Professional:	.9
Others:	.6

Project Description

Objectives:

The humoral immune mechanisms play a major role in the mammalian defense mechanisms against conventional acute viral infections. In contrast, cellular immune mechanisms appear to be important in the defense against the malignant process. The lymphocyte is a major element of the cellular immunity system. It has been demonstrated that the ability of the lymphocyte to respond immunologically is impaired in such diseases as Chronic Lymphocytic Leukemia and Hodgkins disease.

It is planned to examine parameters leading to cellular immunity particularly the inducing factors as well as the resultant malignant cell. The nutritional requirements associated with such cellular responses will primarily be investigated.

Methods Employed:

It has been recently observed that a significant fraction of the lymphocyte population of immunized hosts undergo transformation when stimulated with the specific antigen. This transformation consists of a blastogenesis wherein the small resting lymphocyte becomes metabolically active, enlarges into a lymphoblast form and undergoes mitotic division. The capacity of a lymphocyte population to undergo blastogenesis is considered to be a measure of the immuno-competency of the host. This response can be quantitated by means of standard radioactive labeling techniques. Such a response, among others, will be utilized and investigated in these studies.

Major Findings:

By means of a model system using a non-specific mitogenic stimulant, it has been established that a number of factors, both chemical and physical, are required for the process wherein a metabolically inactive small lymphocyte becomes morphologically transformed and biochemically active. These factors are being delineated and include a large number and variety of nutritional factors as well as environmental factors such as pH and isotonicity. The results of these investigations are in preparation for publication.

Significance to Biomedical Research and the Program of the Institute:

The effects of the oncogenic process in suppressing the immunologic capacities of the host during the course of neoplasia can perhaps be developed as a diagnostic tool as well as providing basic information and techniques as more is learned about the specific processes of the host defense mechanism. Moreover, supplemental therepeutic regimens may be developed.

Proposed Course:

Studies of the biochemical factors which govern lymphocyte reactivity will be continued in order to develop a fuller understanding of the role of the lymphocyte in the immunologic process. The various stages of the immune response will be examined to determine whether they are under specific biochemical control and thus subject to dissociation into discrete biochemical events.

Honors and Awards:

None

Publications:

Hellman, A., Fowler, A. K., Steinman, H. G., and Bovich, P. M.: Lymphocyte transformation with oncogenic and non-oncogenic murine virus. In Vitro (1971)

Fowler, A. K., Hellman, A., Steinman, H. G., and Quatrale, A. C.: The effect of progesterone on mouse lymphocyte transformation. In Vitro (1971)

Fowler, A. K., Hellman, A., Steinman, H. G., and Quatrale, A. C.: Studies on the blastogenic response of murine lymphocytes. I. Quantitative measurements of stimulation by phytohemagglutinin. Proc. Soc. Exp. Biol. Med. (in press)

SUMMARY REPORT

Office of Program Analysis and Communications

Acquisition, management, analysis, and dissemination of Program data within the SVCP are the major activities in PAC. These efforts fall under the two general areas of: (1) data management and statistics; and (2) scientific information storage and retrieval.

Data management and statistics

1. Performs statistical consultant service for scientists in the SVCP program on specific problems of research design, acquisition and handling of experimental data, and its computer automation and analysis.

2. Plans and maintains automated inventories for NIH sponsored serum and tissue specimen collections.

3. Plans and consults with research contractors on automation of their specimen inventories and research data.

4. Promotes compatible automated systems and codes in a multi-institutional plan for a comprehensive clinical and laboratory information storage system for specimens and donors. The goal is a central specimen inventory at NIH to enhance the resource potential of the many large specimen repositories throughout the country.

5. Maintains a system of regular progress reporting from all SVCP research contracts; compiles and distributes summaries of reported progress to program scientists.

Scientific information management

The Information Unit of PAC continued to focus on scientific information retrieval and its dissemination to program scientists. Sources of data are current publications in the field of viral oncology, and other notifications and summaries of current research projects in the field. Major contributions of the Information Unit in FY 1971 were as follows:

1. Bibliographic Service: The automated systems for rapid search, identification, and reference printouts, covering any desired subject in the published literature on viral oncology was maintained.

2. Reading Guide to Cancer Virology Literature: The monthly publication featuring abstracts of current literature on viral oncology, from scientific journals throughout the world,was continued. These abstracts are now incorporated in Carcinogenesis Abstracts; hence, this service will be terminated.

3. Viral Tumorigenesis Report: Indexed summaries of current research projects in the field of viral oncology are compiled and released as semi-annual publications.

4. Report on NCI Support of Cancer Virology Grants: This quarterly report presents an organized summary of updated fiscal data on current NCI research grants in the cancer virology area. The basic information is received from the Program Analysis and Reports Section, NCI.

5. Viral Oncology Contractor Directory: This publication contains pertinent information on all contracts and principal investigators in the Viral Oncology program. Its purpose is to facilitate and expedite communications between staff members and contractors; directory is updated quarterly.

6. Compilation of Journal Instructions to Authors: This displays in one volume the instructions-to-authors from a majority of pertinent scientific journals. It is a reference aid for research investigators in writing papers and also for the secretaries who type them. Compilation will be updated and expanded periodically.

Other responsibilities of the Information Unit are: administration of library facility; collection and distribution of translations of foreign publications in viral oncology; maintenance and lending of recorded tapes on NIH seminars related to viral oncology; and continuous compilation of the SVCP bibliography containing citations to all papers published by viral oncology staff members and contractors.

SUMMARY REPORT

Office of the Coordinator for Ultrastructural Studies

The Virus Studies Section continues to study several aspects of the relationship between oncogenic viruses and their host cells. The problem is approached a) by using the ultramorphologic examination of a number of tumors for the search for viruses; b) by evaluation of different malignancies grown in vitro in respect to the presence or absence of viruses; and c) by studying early events of viral infection which may result in the "neoplastic transformation" of the treated cells. In addition, studies are under way to elucidate the transfer of genetic information from the nucleus to the cytoplasm.

It is important to determine the frequency of the natural occurrence of RNA and DNA containing viruses in human and animal tumors, since both types of viruses may possibly represent causative agents for a number of different malignancies. To our knowledge, to date no systematic attempts to make this determination have been published. Dr. A. J. Dalton in collaboration with Dr. L. Dmochowski, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, and Dr. J. David-Ferreira, Institut Gulbenkian de Ciencia, Oeiras, Portugal, has examined 54 biopsies from human tumors (mostly leukemia, lymphoma and Hodgkins cases) with the electron microscope for the presence of virus particles. All tumors were found to be negative.

Dr. Dalton in collaboration with Dr. J. Szakacs, St. Joseph's Hospital, Tampa, Florida, Dr. U. Heine, Dr. D. Ablashi, and Mrs. J. Kondratick and T. Ben (all NCI) has studied 18 tissue culture isolates from biopsies of human solid tumors for the presence of virus particles. Seventeen cell lines were obtained from these isolates; most lines are fibroblastic in appearance, a few are epithelial-like, and two are suspension cultures resembling the Burkitt lymphoma and human leukemia cultures. One lymphoid cell line, upon co-cultivation with WI-38 cells yielded a cytomegalo virus isolate. The virus was characterized by ultramorphological, biophysical and immunological studies. The other cell lines were negative for virus particles.

Dr. A. Bader, in collaboration with Dr. H. C. Orr, DBS, has examined tissue cultures from a naturally occurring fibrosarcoma in a rattlesnake with the electron microscope for the presence of virus particles. Neither the cells nor tissue culture fluids contained any virus particles.

Dr. Heine in collaboration with Dr. D. Ablashi (NCI) has succeeded in growing Herpes virus saimiri, originally observed in subhuman primates, in different cell lines of human origin (WI-38, primary HEF, WHE). The presence of the virus and its mode of multiplication were demonstrated by virologic and electron microscopic methods.

The study of the early events in viral infection were approached in two separate ways:

Dr. A. Bader in collaboration with Dr. J. Bader (NCI) initiated an investigation to determine the mode of entry of RNA containing tumor viruses into the cell. In addition, by studying the influence of bromodeoxyuridine of chick embryo cells, controls and RSV-infected ones, they contribute to the understanding of the relationship between viral DNA and viral RNA during early infection with an RNA tumor virus. It appears that a viral DNA is made early in the course of infection by avian leuko-sarcoma viruses and that this DNA serves as a genome from which progeny viral RNA molecules are copied.

Dr. Suskind developed quantitative autoradiographic techniques, using tritiated RNA, DNA and Actinomycin D, at the level of light and electron microscopy in combination with ultrastructural examination to study and relate functional and morphologic changes within the nucleus of the intact cell during early stages of infection with the Rous sarcoma virus, Schmidt-Ruppin strain. The results demonstrate differences in both morphology and function in transformed cells with respect to their recovery from inhibition by Actinomycin D. The time interval during which these changes arise has been shown to be about 80 minutes after infection. The findings suggest a causal relationship between the activity of the viral genome and the interval between infection and induction of transformation.

Dr.U. Heine continued studies already in progress correlating cellular particulate material, i.e., particulate nuclear and cytoplasmic structures, with cellular function. Morphologic observations combined with biochemical findings provided evidence that a) 45 S RNA is contained in the fibrillar nucleolar material, b) perichromatin granules may represent the morphologic entities containing messenger RNA and c) the arrest of m RNA transfer from the nucleus into the cytoplasm results in the breakdown of polysomes and their replacement by monosomes.

Dr. Dalton has been responsible for the organization of two monographs in the series "Ultrastructure in Biological Systems" and an Atlas on the ultrastructure of viruses. One hundred twenty-two applications for duty free entry transmission electron microscopes, scanning electron microscopes and vacuum evaporates have been reviewed with recommendations of approval or disapproval to the Florence Agreement Committee, N.I.H.

As a resource for ultramorphologic studies the tissue culture unit established new cell lines of human origin, chick embryo cells for membrane studies and for autoradiographic and ultramorphologic examinations.

In addition to the support of the electron microscope activities, special photographic work was done for the office of the Director (Dr. Baker), the office of the Associate Scientific Director (Dr. Moloney), the office of the Scientific Coordinator for Viral Oncology (Dr. Bryan), and for other sections and branches within NIH (Dr. Manaker, Dr. S. Stewart, Dr. Nomura, Dr. Nirenberg).

The Office of the Coordinator for Ultrastructural Studies has published four papers and presented by invitation six lectures or seminars.

Serial No. NCI-4811
1. Office of the Associate Scientific
   Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on viral replication

Previous Serial Number: NCI-4870

Principal Investigator: Dr. Albert J. Dalton

Other Investigators: None

Cooperating Units: Dr. L. Dmochowski, M.D. Anderson Hospital and
Tumor Institute, Houston, Texas
Dr. Jose David-Ferreira, Instituto Gulbenkian
da Ciencia, Oeiras, Portugal
Dr. Jeno E. Szakacs, St. Joseph's Hospitl, Tampa, Florida

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

Examination of biopsy material and tissue culture isolates from human neoplasms with the electron microscope for the presence of virus particles;

(a). Analysis of biopsy material from human neoplasms with the electron microscope to determine incidence of cases positive for virus. (Instituto Gulbenkian da Ciencia, Oeiras, Portugal and M.D. Anderson Hospital and Tumor Institute, Houston, Texas.)

(b). Electron microscopic analysis of tissue culture isolates of human solid tumors to determine how often evidence of the presence of virus is found. (St. Joseph's Hospital, Tampa, Florida)

Major Findings:

A. In Portugal 44 biopsies of human leukemic nodes and lymphomas were examined in detail. On the average 4 different areas of each biopsy were examined and from these areas 4 different grids were analyzed. In no instance was virus found. During the stay in Portugal a talk was given on viruses and cancer at the meeting of the Portugese-Spanish Academy of Sciences in Lisbon. A talk was also given on the same subject at the University of Perugia, Italy. A trip was taken to England to make judgments on the new analytical electron microscope developed in part by AEI. This instrument has a fascinating future in that it is possible to examine minute structures with a transmission electron microscope and then to analyze the same structures quantitatively by electron probe analysis with an associated digital computer for elements from magnesium to the heavy metals. The visit to the high-voltage electron microscope installation was somewhat disappointing in that the instrument was not being used primarily for biological studies, and the tilt stage was not functioning. Cut film was used for photography and this film buckled in the vacuum making it impossible to close the plate cover; thus requiring frequent repeats of exposures. On the basis of this experience it is not recommended that this particular model be considered for installation at NIH. 1/3 man year.

B. M. D. Anderson Hospital and Tumor Institute.
PHS-45-65-604. In addition to the cases studied in Portugal, ten additional biopsy specimens have been examined at NCI. All ten were negative for virus. 1/6 man year.

C. Ultrastructure of cells and viruses in tissue culture isolates from human solid tumors. St. Joseph's Hospital, Tampa, Contract NIH-69-2074. Twenty-two of these isolates have been examined by electron microscopy and only one of these was found to contain virus. This was #13 in which a cytomegalo virus was demonstrated by rescue using WI-38 cells. 1/6 man year.

D. Co-editor for and contributor to the monograph on the Ultrastructure of Viruses. List of contributors:

Picorna viruses: G. Godman, Columbia University.
RNA viruses: S. Dales, Public Health Research Institute of the City of New York.
Arboviruses: N. Higashi, Kyoto University.
Myxo & Paramyxo viruses: P. W. Choppin and R. C. Compans, Rockefeller University.

Rhabdo viruses: K. Hummeler, Childrens Hospital, Philadelphia.
Avian leukoses: J. Beard, Duke University.
Murine leukemia & sarcoma: W. Bernhard, Institute Cancer Research, Villejuif, France.
MTA: D. Moore and N. H. Sarkar, Institute for Medical Research.
Corona: L. S. Oshiro, University of California, Berkley.
Arena: A. J. Dalton, National Cancer Institute.
Australia antigen: M. D. Hoggan, National Institutes of Health.
Pseudo viruses: F. Haguenau, College de France, Paris.
Pox viruses: S. Dales, Public Health Research Institute of the City of New York.
Herpes: B. Roizman, University of Chicago.
Adeno: R. Horne? (Postal strike), University of Norwich, England.
Papova: A. Howatson, University of Toronto.
Parvo(picodna): H. D. Mayor, Baylor University.

(T. Anderson, Institute for Cancer Research, Philadelphia, Pa.
Phage: (E. Kellenberger, Geneva, Switzerland.
(F. Eiserling, University of California, Los Angeles.

(4) Advisory Board to monograph series "Ultrastructure in Biological Systems." A. J. Dalton and F. Haguenau, eds.

L. E. Benedetti, Institute Molecular Biology, Paris. Biological Membranes.
R. Buvat, Lab. Biology and Cytology, Marseille, Plants.
S. Dales, Public Health Research Institute of the City of New York. Viruses.
P. Drochmans, Free University of Brussels, Cytology and cancer.
P. Favard, Lab. of Atomic Synthesis. Paris. Molecular biology.
D. Fawcett, Department of Anatomy, Harvard University. Ultrastructure of animal cells - spermiogenesis.
H.Ris, Dept. of Zoology, University of Wisconsin, Nucleus.

E. Administrative.

(1) Consultation on ultrastructure of cells and viruses - inhouse and extramural.

(2) Site visits involving (a) purchase of electron microscopes and ancillary equipment: (b) Ultrastructural studies on cells and viruses.

(3) Florence Agreement Committee - review of applications for duty free entry of electron and scanning microscopes and vacuum evaporators.

(4) SVCP. Project officer on 3 contracts.

(5) BABC meetings.

(6) Review of manuscripts as associate editor of Cancer Research and Journal of Ultrastructure Research. One-third man year.

Significance to Biomedical Research and the Program of the Institute:

Within practical limits it is important to determine how often virus can be identified in biopsy specimens from human leukemia and lymphoma. Up to now no serious attempt to make this determination has been published. It is also important to the program to determine how often virus is encountered in human tissue culture isolates where viruses are relatively free of immunological restraints.

Publication of the atlas on the ultrastructure of viruses will fill a long-felt need of virologists in general as well as those working in the general field of cell and virus ultrastructure and particularly oncogenic viruses.

Proposed Course:

To continue electron microscopic analysis of human biopsy material until a satisfactory significant and scientifically acceptable number of cases has been surveyed.

The number of successful tissue culture isolates containing virus is small (1 out of 22). It is planned to continue this effort until the kinds of virus to be found or rescued by this approach can be determined.

In addition to the atlas on the ultrastructure of viruses, two other monographs are in the planning stage, one on the ultrastructure of heart muscle and one on the ultrastructure of the anterior hypophysis.

Honors and Awards:

Invited by the Gulbenkian Foundation for four months as a guest worker at the Instituto Gulbenkian da Ciencia, Oeiras, Portugal.

Invited to give a lecture on viruses and cancer at the meeting of the Portugese-Spanish Academy of Sciences, Lisbon, April, 1970.

Invited to give a lecture on viruses and cancer at the University of Perugia, Italy, May, 1970.

Publications:

Albert, D. M., Dalton, A. J., and Rabson, A. S.: Microtubules in retinoblastoma. Am. J. Ophthal. 69: 296-299, 1970.

Serial No. NCI-4812
1. Office of the Associate Scientific Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Virus-host Cell Interactions

Previous Serial Number: None

Principal Investigator: Dr. Ursula Heine

Other Investigators: Dr. D. V. Ablashi
Dr. R. A. Maca
Dr. A. J. Dalton

Cooperating Units: Outside NIH
Dr. R. A. Bonar, Dr. L. Sverak, Duke University Medical Center, Durham, N. C.
Dr. Jeno Szakacs, St. Joseph's Hospital, Tampa, Florida, NIH-69-2074

Man Years:

Total:	2
Professional	1
Other:	1

Project Description

Objectives:

1. To study and identify selected viruses in relation to their host cells by ultrastructural and other biological methods.

2. To study the relationship between structure and function of cellular components before and after infection with tumor viruses.

Methods Employed:

Suitable virologic methods and electron microscopic techniques were used for cell studies at the ultrastructural level. For the investigations of cellular functions biochemical methods (radioisotope tracers, cell fractionations, RNA extractations, gradient sedimentations and spectrophotometry)were used in addition to E M techniques.

Major Findings:

A. Tissue culture isolates from human solid tumors have been studied with the electron microscope to determine the presence or absence of viruses in these isolates. A continuous cell line of lymphoid blast cells was obtained from a biopsy of a recurrent paraganglioma with lymphoid infiltration. The blast cells contained unique tubular structures in cytoplasmic areas but no virus. By cell-to-cell contact with WI-38 cells a Herpes-type virus was rescued. Ultrastructural, biophysical and immunological studies characterized the virus as a cytomegalovirus. Tests against Herpes hominis I and II and E-B virus were negative. The evidence suggests that the presence of cytomegalovirus in lymphoid cells may stimulate continuous growth in tissue culture. This work was done in collaboration with Drs. Dalton, Szakacs, and Ablashi.

B. In collaboration with Dr. Ablashi the propagation of Herpes virus Saimiri was studied in different cell strains of human and subhuman origin. Morphologic evidence was given for the presence and the maturation of the virus in WI-38, AGMK and OMK cells. Although belonging to the Herpes group this virus exhibits unique characteristics in regard to its completion inside the cell.

C. Viral and morphologic studies were carried out on hepatomas in mice of strain C3H-A^{vy} and on a cultured hepatoma. This work was done in collaboration with Drs. R. A. Maca and R. Manaker, Microbiology Section, VBB. Light and electron microscopic studies revealed highly differentiated epithelial cells in primary and subcutaneously transplanted tumors, which resembled in many aspects their cells of origin, the hepatocytes in normal liver. Epitheloid cells from these tumors grown in vitro were morphologically dissimilar and appeared to be dedifferentiated. Occasionally immature "C"-type particles have been observed in primary and transplanted hepatomas and viral particles were frequent in cultures of hepatoma tissue. The virus is morphologically characteristic of the murine leukemia group and contains the group specific antigen, as determined by the complement fixation test. This project is completed.

D. The investigations of the influence of the antibiotic Toyocamycin on HeLa cells, which inhibits selectively the cleavage of 45 S ribosomal RNA in mammalian cells, were continued. Morphologic studies gave evidence of specific structural changes in the nucleoli of HeLa cells under the influence of the drug, which are expressed by an excessive accumulation of fibrillar material. But small numbers of granules, the other prominent component of a normal nucleolus, remained present in the treated nucleoli. Therefore, further attempts to clarify the relationship of the two nucleolar components were desirable. A pretreatment with supranormal temperatures, which stimulates an intensive synthesis of nucleolar particulate material, followed by a recovery in the presence of Toyocamycin, resulted in the reconstruction of the nucleolus with above normal amounts of 45 S RNA, visualized by the complete absence of nucleolar granules. Thus, further and convincing evidence is obtained suggesting that the 45 S RNA precursors are located in the fibrillar part of the nucleolus.

In the course of these studies an intensive investigation of the effects of supranormal temperatures on cells in vitro was carried out in collaboration with Drs. Sverak and Bonar, Duke University, Durham, N. C. Synthesis and subsequent conversion of the 45 S ribosome precursor RNA in HeLa cells were each inhibited to a certain degree at elevated temperatures; whereas the amount of RNA in the 30 S to 4 S region was relatively more pronounced at the same time. Morphologic observations were consistent with biochemical findings. They provided evidence that
a) 45 S RNA is contained in the fibrillar nucleolar material,
b) perichromatin granules may represent the morphologic entities containing messenger RNA, and c) the arrest of m RNA transfer from the nucleus into the cytoplasm results in the breakdown of polysomes and their replacement by numerous monosomes.

An interesting implication of this work is worthwhile mentioning. It has been known that high temperature may block cancer development and a therapy on this basis has been developed. In view of the work presented it appears to be evident that rapidly proliferating cells may be more affected by a temperature shock than non-growing cells.

Significance to Biomedical Research and the Program of the Institute:

A. Efforts to detect viruses in human tumors as a causative agent for unlimited growth are of utmost importance for the etiology of human cancer. The rescue of a cytomegalovirus from a continuous human lymphoid cell line free of detectable virus particles contributes in this respect, since it suggests that cytomegalovirus, as well as E-B virus, may stimulate cells to continuous proliferation. The presence of characteristic cytoplasmic inclusions of tubular array in these rapidly growing lymphoid cells may represent a characteristic and easily identifiable marker for viral infection.

B. Herpes virus Saimiri with its unique characteristics, i.e., the failure of its detection in vivo and its ready rescue in vitro, serves as an excellent experimental model for the study of human cancer. It is of interest to investigate in greater detail the behavior of this virus in different systems and compare it with known non-oncogenic DNA viruses.

C. In an experimental model, a mouse hepatoma grown in vivo and in vitro "C"-type virus particles were identified. These findings add to the already known fact of the ubiquity of the murine RNA viruses.

D. In studying the action of the antibiotic Toyocamycin on mammalian cells, further understanding of the transfer of genetic information from the nucleus into the cytoplasm has been obtained.

Proposed Course:

A. We are expanding our investigations for the search of Herpes Virus Saimiri in vivo by studying different organs and tumors of infected monkeys with the electron microscope. This material will be obtained from Dr. Ablashi, Lymphoma and Leukemia Branch, NCI. In collaboration with Dr. A. J. Dalton the behavior of this virus in a number of host cells will be investigated. A comparison of the host-cell relationship of this virus with that of other oncogenic and non-oncogenic DNA viruses (H. simplex, E-B virus, Cytomegalovirus) is initiated.

B. The studies concerning the relationship between structure and function of cellular components before and after infection with tumor viruses will be pursued. Toyocamycin with its unique influence on cellular metabolism can be used to direct events on the molecular level. Basic studies on the influence of this antibiotic on the metabolism of normal cells are completed and the knowledge obtained is now applicable to virus-infected cell systems.

In addition, a contract with Dr. G. S. Beaudreau, Oregon State University, entitled "Study of the replication and function of nucleic acids from oncogenic virus" has been initiated.

Honors and Awards:

Dr. Heine presented a paper entitled. "Cytomegalovirus in a continuously growing tissue culture isolate" at the 2nd Medical Education and Research Conference in Tampa, Florida, Feb. 2, 1971.

Dr. Heine presented a seminar entitled "Nucleolar changes in HeLa cells under the influence of Toyocamycin" to the 1st Institute of Pathology, Medical University, Budapest, Hungary, Sept. 8, 1970.

Publications:

Heine, U. I.: The influence of supranormal temperatures on the nucleolus of HeLa-S_3 cells exposed to the antibiotic toyocamycin. Archiv für Geschwulstforschung 36: 111-122, 1970.

Heine, U., Sverak, L., Kondratick, J., and Bonar, R. A.: The behavior of HeLa-S_3 cells under the influence of supranormal temperatures. J. Ultrastructural Research 34: 375-396, 1971.

Maca, R. A., Heine, U., and Manaker, R. A.: Viral and morphologic studies on hepatomas in mice of strain C3H-A^{vy} with observations of a cultured hepatoma. Archiv für Geschwulstforschung 36: 213-230, 1970.

Heine, U., Kondratick, J., Ablashi, D. V., Armstrong, G. R., and Dalton, A. J.: Ultrastructural and biological properties of a cytomegalovirus rescued from a human paraganglioma. Cancer Research, in press.

Heine, U., Ablashi, D. V., and Armstrong, G. R.: Morphologic studies on Herpes Virus Saimiri in subhuman and human cell cultures. Cancer Research, in press.

Serial No. NCI-4813
1. Office of the Asssociate Scientific Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) Adsorption and Penetration of RNA-containing Tumor Viruses into Chick Embryo Cells

(B) Studies of the Replicative Genome of RNA-containing Tumor Viruses

(C) Investigation of a Naturally Occurring Fibrosarcoma in a Rattlesnake for the Presence of Virus Particles

Previous Serial Number: None

Principal Investigator: Dr. Artrice V. Bader

Other Investigators: Dr. John P. Bader
Dr. Henry C. Orr

Cooperating Units: Inside NIH
Cell Growth Regulation Section, Chemistry Branch, NCI
Cell Biology Section, Laboratory of Virology and Rickettsiology, Division of Biologics Standards

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

(A) To determine the mode of entry of RNA containing tumor viruses into cells.

Serial No. NCI-4813

1. Office of the Associate Director for Viral Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) Adsorption and Penetration of RNA-containing Tumor Viruses into Chick Embryo Cells.

(B) Studies of the Replicative Genome of RNA-containing Tumor Viruses.

(C) Investigation of a Naturally Occurring Fibrosarcoma in a Rattlesnake for the Presence of Virus Particles.

Previous Serial Number: None.

Principal Investigator: Dr. Artrice V.Bader

Other Investigators: Dr. John P. Bader (NCI-4765)
Dr. Henry C. Orr (DBS-83)

Cooperating Units:

Cell Growth Regulation Section, Chemistry Branch, NCI
Cell Biology Section, Laboratory of Virology and Rickettsiology, Division of Biologics Standards

Man Years:

TOTAL: 2
PROFESSIONAL: 1
OTHER: 1

Project Description

Objectives:

(A) To determine the mode of entry of RNA containing tumor viruses into cells.

(B) To investigate the possibility of a DNA replicative genome for RNA tumor viruses.

(C) To determine if virus particles were present in a cell culture line originating from a naturally occurring tumor derived from a reptile.

Methods Employed:

(A) Electron microscopy, phase microscopy, cell culture, differential centrifugation, density layering.

(B) Electron microscopy, cell culture, radioactive labelling, density equilibrium centrifugation, polyacrylamide gels.

(C) Cell culture, electron microscopy.

Major Findings:

(A) Chick embryo cells established in culture are employed for studies on the early infection of RNA containing tumor viruses. All cell lines used in these studies were checked by the Rous interference (RIF) test and electron microscopy to preclude the presence of leukosis virus deriving from congenitally infected embryos. These cells are exposed to a mixture of Rous sarcoma virus and Rous associated virus, fixed at different time intervals and processed for observation with the electron microscope. These cells were then examined to see if the actual mode of viral entry could be determined. The results indicated that the use of the intact cell was quantitatively insufficient to resolve the problem and, therefore, different membrane isolation procedures were employed in an effort to obtain the best technique for the isolation of plasma membranes from chick embryo cells. RNA tumor viruses were added to the cells before they were subjected to the procedures for removal of membranes, i.e. homogenization, differential centrifugation and density layering. The experiments were monitored by phase contrast and electron microscopy. Membrane preparations have been obtained which contain plasma membranes but also other smooth membranes. Current work is being directed towards a procedure which will provide only the plasma membrane in the final fraction.

(B) Virus particles obtained from cell cultures which had been treated with bromodeoxyuridine (BrdU) and untreated cell cultures were counted in the electron microscope and examined for morphological changes. A negative staining technique, using latex particles at a known concentration, was employed to obtain a quantitative particle count. Only a slight difference was found in the number of physical particles produced by BrdU-treated and untreated cells. There was no difference in morphology of particles produced by BrdU-treated cells and those of control cultures. BrdU-treated cells transformed to the same extent as non-treated cells. The synthesis of group specific antigen was likewise unaffected by BrdU treatment. However, specific infectivity of virions produced by BrdU cultures was substantially less than that of the controls. Since BrdU had no effect on cell growth and mitoses during the experimental period, these results indicate that a new viral DNA is made and that this new DNA serves as a genome from which progeny viral RNA molecules are transcribed.

(C) A tissue culture cell line was established from a naturally occurring tumor in a rattlesnake. Pellets of cells and fluids obtained from the cultures were processed for thin sectioning and observed with the electron microscope. Examination of these cells and fluids did not reveal the presence of virus particles. The cells were not typical of fibroblastic cells in that the majority of them had endoplasmic reticulum more characteristic of cells secreting a product.

Proposed Course:

(A) The use of isolated membranes will be used to complement the data obtained from intact cells which should serve as a better model than the intact cell for an analysis of the adsorption and penetration of RNA-containing tumor viruses.

(B) There are no current plans for the extension of the electron microscope phase of this project.

(C) Project currently concluded.

Significance to Biomedical Research and this Institute:

(A) Compared to the morphology of virus completion, i.e. budding and completion of virions at the cell membrane, which has been well documented, the entry of RNA-containing tumor viruses has not been elucidated. The mode of entry of RNA-containing tumor viruses is important to an understanding of the infectious cycle of these viruses and their role in oncogenesis.

(B) Earlier studies have shown the requirement of DNA synthesis for the reproduction of RNA tumor viruses. The results of this study indicate that a viral DNA is made early in the course of infection by avian leuko-sarcoma viruses and this DNA serves as the genome from which progeny viral RNA molecules are copied. The data obtained from this study support the hypothesis of reverse transcription, wherein the viral RNA serves as a template for the synthesis of a new viral DNA. This new viral DNA serves as the template for progeny viral RNA. More recent reports demonstrating the RNA-dependent DNA polymerase associated with the RNA containing tumor viruses provides additional evidence for a DNA replicative genome for RNA containing tumor viruses.

(C) It is known that RNA-containing tumor viruses occur in nature in avian, murine and feline species. There has been one previous report describing these particles in a pokilothermic animal. The particles were found in a spleen line obtained from a snake (Russell viper) which had a naturally occurring myxofibroma. They were not found in cell cultures derived from the original tumor. Documentation of the presence or absence of RNA containing tumor viruses in a different species of a pokilothermic organism will provide additional information pertaining to the natural occurrence of RNA-containing tumor viruses. In addition, it will possibly provide an additional model for the study of the viral etiology of cancer.

Publications:

Bader, J. P., and Bader, A. V.: Evidence for a DNA replicative genome for RNA-containing tumor viruses. Proc. Nat. Acad. Sci. 67: 843-850, 1970.

Bader, J. P., Brown, N. R., and Bader, A. V.: Characteristics of cores of avian leuko-sarcoma viruses. Virology 41: 718-728, 1970.

Serial No. NCI-4814
1. Office of the Associate
Scientific Director for Viral
Oncology, Etiology
2. Ultrastructural Studies Unit
3. Bethesda, Ma yland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Autoradiographic and Ultrastructural Studies of the Nucleus of Chicken Fibroblasts during the Eclipse Phase of Infection with Rous Sarcoma Virus

Previous Serial Number: None

Principal Investigator: Dr. R. Gerald Suskind

Other Investigators: Dr. Francoise Haguenau, College de France, Paris
Dr. Giancarlo F. Rabotti, College de France, Paris
Dr. Susan C. Michelson-Fiske, College de France, Paris

Cooperating Units: Laboratoire de Medécine Experimentale, College de France, Paris

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description

Objectives:

The intracellular mechanism of infection with Rous sarcoma virus during the eclipse phase and the events within the nucleus leading to "neoplastic" transformation is under investigation using quantitative light and electron microscopic autoradiographic techniques in conjunction with sequential assays of ultrastructural changes within nuclei.

Methods Employed:

The methods employed were tissue culture of chick fibroblasts; titration of Schmidt-Ruppin Rous sarcoma virus; radioactive tracer studies using tritiated RNA and/or DNA precursors as well as tritiated Actinomycin D; quantitative autoradiographic techniques at the level of the light and electron microscope in combination with ultrastructural examinations.

Major Findings:

Consistent differences between nucleoli of uninfected, newly infected and transformed cells were found with respect to their reaction to a pulse exposure of Actinomycin D, both by quantitative autoradiography and by morphologic grading of nucleoli. Ultrastructurally these have been localized to the fibrillar portion. In addition, the rate of recovery from inhibition by Actinomycin D is parallel in transformed and newly infected cells over a period of 10 hours, but is about twice as fast in uninfected cells. A constant time differential was observed between the functional activity of nucleoli of transformed cells and those of newly infected cells, the latter synthesizing the same relative amount of RNA as the former within 80 min. of infection. At the end of the period of infection this same time interval between control cells, newly infected and transformed cells was observed in the rate of morphologic recovery of nucleoli. It is suggested by these findings that this may represent the time interval during which induction of the transformed state by the viral genome occurs.

The hypothesis that these observations might be the result of quantitative or conformational changes induced by the viral genome in the binding sites of Actinomycin D on nucleolar DNA was tested by measuring the distribution of tritiated Actinomycin under these experimental conditions. Although no differences in the initial binding of Actinomycin D to nucleoli or nuclei of transformed cells were detected, it was found that the rate of release of Actinomycin D label from nucleolar sites is significantly slower in newly infected and transformed cells than in uninfected cells within the time of observation. No significant differences in the labeling of non-nucleolar areas of the nucleus were found. We interpret these findings as evidence for the formation of new and competing binding sites for Actinomycin D in nucleolar areas as a result of infection. This is unlikely to be the result of new DNA synthesis as our data so far indicate continued inhibition in infected cells and complete absence of mitotic activity for 24 hours.

Significance to Biomedical Research and the Program of the Institute:

A quantitative tool for studying and relating functional and morphologic changes within the nucleus of the intact cell during very early stages of infection with an oncogenic virus has been developed. The results obtained thereby have demonstrated differences in both morphology and function in transformed cells with respect to their recovery from inhibition by Actinomycin D. The time interval during which these changes arise has been shown to be about 80 minutes after infection.

These findings suggest a causal relationship between the activity of the viral genome and the interval from infection to the induction to the transformed state. It has been shown that these phenomena are related to a lower sensitivity of transformed cells to the inhibitory action of Actinomycin D, and that this results from the formation of new and competing binding sites for Actinomycin D in nucleoli of infected cells. These findings have heuristic implications for determining the sites of attachment and manner of replication of the viral genome.

Proposed Course:

The possibility that the described biological phenomena may be a clue to determining both the site of attachment of the viral genome and to the time interval during which the transformed state is induced is suggested by these experiments. Further experiments to clarify the basis of these observations, particularly with respect to DNA synthesis, the species of RNA synthesized and the effect of infection with a non-transforming virus are required and are either in progress or planned.

Honors and Awards:

Dr. Suskind presented a paper entitled: "Autoradiographic and Ultrastructural Studies of the Eclipse Phase of Infection with Rous Sarcoma Virus, Schmidt-Ruppin Strain, in Chicken Fibroblasts" at the Annual Meeting of the American Association for Cancer Research, April 8, 1971, Chicago, Illinois.

Publications: None.

SUMMARY REPORT

VIRAL LEUKEMIA AND LYMPHOMA BRANCH
July 1, 1970 - June 30, 1971

The Viral Leukemia and Lymphoma Branch conducts research designed to elucidate the role of viruses in the etiology of human neoplasms, particularly leukemias, lymphomas and sarcomas. A variety of scientific approaches are used which provide a broad base of knowledge applicable to the identification and isolation of human oncogenic agents and the prevention or control of the disease as it occurs in man. More specifically, the Branch encompasses a range of scientific disciplines including molecular biology, genetics, immunology, biochemistry, pathology and cell culture techniques.

The Section of Molecular Biology seeks to obtain comprehensive knowledge of the biology and biochemistry of sarcoma and leukemia viruses and conducts quantitative studies on the interaction of oncogenic viruses and cells to determine the mechanisms of viral replication and cellular transformation at the molecular level. The Section of Viral Pathology studies the biology of the RNA-containing oncogenic viruses using a combination of in vitro and in vivo techniques; thus, animal species and tissue culture systems are infected with leukemogenic and sarcomagenic viruses to elucidate the role of the host in viral propagation and tumor development. The Section of Immunology examines the antigenic nature of oncogenic viruses and the induced tumors as well as the immune response of the host to viral infection and tumor development. The Section of Tumor Viruses is concerned with defining in detail the biological and biochemical properties of tumor viruses in order to understand how they may be applied to the search for human tumor viruses. A "helper" assay to "rescue" oncogenic virus information is currently being applied to human cell systems. The Section of Genetics is concerned with genetic factors of both the tumor virus and the host it infects that are involved in the oncogenic process. Particular emphasis is placed on viral genes involved in oncogenesis and cellular "susceptibility" genes, particularly those genes of man that predispose individuals to the development of cancer. The Office of the Chief coordinates the research of the various sections by recognizing the scientific freedom of the individual investigators. The office is responsible for establishing collaborative efforts with investigators in other areas of NIH and elsewhere such that information derived from studies within the Branch is constantly being applied in investigations leading to a better understanding of the etiology of human neoplasia.

One of the most important findings in the tumor virus field in the past year has been the discovery that certain RNA tumor viruses carry, in the virus particle itself, enzymes that are capable of transcribing the viral RNA back into DNA. These observations lead to the possibility of using extremely sensitive biochemical probes to search for evidence of viral etiology of cancers, and especially, cancers in man. Some of the potential applications to the etiology and control of human cancer are:

1. The use of synthetic DNAs produced from the viral RNA to search for complementary RNA in human tumors by DNA-RNA hybridization techniques.

2. The use of highly effective synthetic templates and optimal enzymatic conditions to search for tumor virus specific enzymes in human tumor cells.

3. The use of the tumor virus polymerase and human tumor cell polymerase to screen for specific inhibitors that may be used as possible chemotherapeutic agents (e.g., rifampicin derivatives prevent the synthesis of DNA from RNA by the viral polymerase).

4. The use of specific antiserum prepared against the purified viral enzymes to identify individuals that have been exposed to the viral enzyme. It is reasonable to expect that the antibodies to viral specific proteins may persist for much longer periods than the virus itself would persist.

All of the above approaches are being actively followed by members of the Viral Leukemia and Lymphoma Branch. One of the major concerns was to answer whether the enzyme is specific for tumor viruses and whether it is specific for tumor cells. All "C" type and "B" type oncogenic viruses tested have been found to have DNA polymerase as detected both by an endogenous reaction and by a synthetic polymer-stimulated reaction, using such templates as poly rA·rU, poly rI·rC and poly rA·dT. The species that have polymerase-containing virions include the mouse, rat, hamster, cat, chicken and monkey. A variety of non-oncogenic viruses that, like the oncogenic viruses, develop by budding from the plasma membrane, have shown no evidence of this enzyme activity; these include influenza, Newcastle Disease Viruses, reovirus, Vesicular Stomatitis Virus, poliovirus, respiratory syncytial virus, Sendai virus and such common infectious viruses of man as measles, mumps, and rubella. This survey indicated a very strong association between presence of the enzyme and oncogenic potential of the virus. However, two exceptions were found. The first is visna virus, a virus of sheep which produces a chronic, progressive, neurologic disease but has, heretofore, not been associated with malignancies in sheep. The second major exception are the group of "foamy" viruses. These can be commonly isolated from monkeys, cattle, and cats and have also been isolated from hamsters. These viruses, though latent, have not yet been associated with any disease. Studies in this Branch by Drs. Edward Scolnick and Stuart Aaronson in conjunction with Dr. Wade Parks of the Viral Carcinogenesis Branch have established that the foamy viruses are RNA containing viruses that, like the tumor viruses, appear to replicate via a DNA intermediate. RNA dependent DNA polymerase has been found in the simian "foamy" viruses and also the bovine, hamster and feline "foamy" viruses. The finding of this enzyme in this long neglected class of viruses raises the possibility that they may be involved in the oncogenic process. Since they are extremely common in primates, a study of their biology, biochemistry and epidemiology appears to be indicated. Visna and "foamy" viruses at the moment, then, appear to be exceptions to the rule that only tumor viruses contain this enzyme; however, since neither has been seriously tested for its oncogenic capacity, this question remains open.

Work in the Viral Leukemia and Lymphoma Branch established that the enzyme is located in the nucleoid of the virion and the antiserum directed against the polymerase could inactivate the enzymatic activity. The use of purified gamma globulin to inhibit the rate of the enzymatic reaction appears to offer an extremely sensitive test for detecting antibody and the combined disciplines of immunology and biochemistry are being utilized to see if this approach can be extended to detection of antibodies to tumor virus enzymes in human serum.

Other Research Developments in the Branch

The development of continuous, contact-inhibited mouse cell lines from Balb/c and NIH/Swiss embryo cells has provided excellent model systems for study of the effects of tumorigenic viruses both *in vitro* and *in vivo*. These cell lines are supplied to numerous investigators throughout the world, and are becoming the standard cell lines for biochemical and biological investigations of cellular growth control mechanisms.

A new type of MSV-transformed cell, the nonproducer cell, has been discovered. These cells are morphologically transformed and are highly tumorigenic, yet they lack all the known antigens of the murine sarcoma-leukemia complex. The sarcoma genome can be readily rescued by the addition of "helper" leukemia virus. These nonproducer cells provide a very good model for cancer in man. Methods for the detection of virus specific information in these cells are in progress. Those methods that are most sensitive and most specific will be applied to the study of human sarcoma cells.

The isolation of cloned cell lines infected with MSV in the absence of detectable MuLV (S^+L^- cells) has permitted the study of the defectiveness of MSV. Superinfection of S^+L^- cells with MuLV resulted in a logarithmic rise in titer of both MSV and MuLV in the supernatant, beginning 12 hours after infection and becoming maximal at 72 hours. While MSV was not produced without the simultaneous release of progeny MuLV, an excess of MSV was produced early after superinfection. This MSV excess permitted the isolation of MSV from MuLV. Application of these preparations of MSV to 3T3 cells resulted in the production of S^+L^- cells. Inhibition of DNA synthesis during the period of eclipse between superinfection and the release of MSV and MuLV resulted in a decrease in production of both virus types.

A potent murine sarcoma virus (MSV) isolated from a spontaneously arising mouse tumor was found to have properties similar to those other well characterized strains of MSV. However, after hamster passage, the tumors so induced produce a virus that is antigenically related to the mouse virus but is apparently non-infectious. The sarcoma genome can be rescued from the hamster tumor cells. The hamster derived virus contains 70S RNA and has a density of 1.16 but is deficient in RNA-dependent DNA polymerase, and does not contain gs-3. Preliminary data suggests that the virus is a sarcoma virus produced in the absence of detectable leukemia virus of either murine or hamster origin.

Poly I • Poly C, a potent interferon inducer, has two actions in MSV tumor induction. Pretreatment with small doses greatly enhances MSV induced tumors, while repeated injections of large toxic doses suppress tumor formation. Poly I•C and MLV enhance the appearance of antibodies to RNA in New Zealand mice. Pre-immunization with Poly I•C retards the appearance of natural and MLV induced antibodies. The data indicates that the mechanism of production of antibodies to RNA following inoculation of MLV is similar to that operative in the natural state. DEAE-dextran enhances MSV both *in vivo* and *in vitro*. In both cases the enhancement is approximately tenfold. The *in vivo* effect is seen both after local and systematic administration of DEAE-dextran.

The relationship between age, autoimmune status and susceptibility to MSV has been studied in New Zealand mice of various ages. While old and young NZ mice are equally susceptible, old mice have considerable difficulty in regressing their tumors. This effect is seen prior to the appearance of autoimmune disease, indicating that immune depression precedes the appearance of autoimmunity. Weanling New Zealand mice can regress MSV induced tumors at a considerably earlier age than other strains, indicating early appearance of immunologic competence.

Improved biochemical methods for fractionation and separation of murine leukemia virus antigens have been developed. Both major and minor antigens have been found. Methods have also been improved to purify and separate the antiviral antibodies from other serum antibodies and proteins. The goal of these studies are to produce monospecific antibodies to each of the viral structural proteins and each of the viral enzymes.

Efforts continue toward the isolation of viruses or antigens specific to human and feline breast cancer using the murine MTV model system. Two human and two feline cell lines are under study. Serologic tests (HA, HAI, ID and neutralization) were adapted this year to the identification and quantitation of feline leukemia virus and antibodies. Tests to monitor cytotoxic antibody and cellular immunity were also developed. Experiments are in progress to develop cellular hyperimmunization protocols against oncogenic viruses and tumor antigens. Tests of 375 patient sera for HAI antibodies against FeLV, MTV and RLV showed that about 20% of the patients react with these viruses. There was no pattern of association of antibodies to any virus and a given diagnosis. Yet, certain human sera appeared to contain specific antibodies against one or more of the viruses tested; the significance of these findings is being actively studied.

Methods have been developed which greatly increase the sensitivity of detection of the viral RNA dependent DNA polymerase using synthetic templates with manganese as the divalent cation. It has been possible to disrupt the tumor virus and to partially solubilize it. Polymerase activity is associated with the internal core of the virus. Certain antisera to RNA tumor viruses inhibit the polymerase and studies to date indicate that these sera contain an antibody which inhibits the viral polymerase of several mammalian C-type RNA viruses but do not inhibit the polymerase of avian C-type RNA viruses or the polymerase of the mammary tumor virus.

Syncytium forming ("foamy") viruses of several species including the primate, bovine, hamster and feline have been found to contain an RNA dependent DNA polymerase. Studies to define their pathologic, antigenic and biochemical characteristics are in progress. Attempts to find isolates of such viruses from human tissues are also under way; since they are so common in other species it is reasonable to suspect that there are human polymerase-containing "foamy" viruses.

Recently developed biochemical methods to study the viral RNA dependent DNA polymerase in virions have been extended to search for evidence of such enzymatic activity in cells using the model system of MSV transformed and normal Balb/3T3 cells. It has been possible to detect enzyme activity in normal as well as transformed Balb/3T3. In addition, normal human fibroblasts and normal human lymphocytes contain polymerase activity. The relationship between the enzymes in normal cells, tumor cells and tumor viruses provides the major focus for present studies.

Inhibitors of the tumor virus polymerase are being tested for their ability to inhibit virus replication and transformation. Certain rifampicin derivatives have been found to be 50-100 times more active than N-demethyl rifampicin in _in vitro_ assays using the purified mouse leukemia virus polymerase.

Eight patients with acute leukemia and their identical twins were evaluated by a variety of techniques. Lymphocyte cytotoxicity, mixed lymphocyte culture and skin testing were used to determine whether cellular immunity factors could identify a tumor specific antigen, and whether there was evidence that this antigen caused immune reactivity in family members and controls. Cells from the patient and his identical twin were used as sources of antigens directly and materials were also placed in tissue culture. Skin fibroblasts from family members were tested to determine whether any genetic factors could be associated with acute leukemia. Electron microscopy and immunofluorescence, using antisera against Rauscher and feline leukemia virus, would be utilized to determine whether any of the antigens detected by cellular immunity tests were related to any known animal leukemia viruses. An antigen which appears to be a leukemia associated antigen was detected in five of seven sets of identical twins.

The study of identical twins will continue in an attempt to more carefully define the specificity of the leukemia associated antigens and the immune response of humans to these antigens. New tests will be applied to the study of twins as they are developed. Since these families are ideal for the study of genetic and immunological susceptibility, more identical twins will be sought for study.

A study of the effect of UV irradiation of the DNA tumor virus, SV40, on the survival of its viral functions in mouse and human cells has revealed that the viral functions for T antigen and transformation show comparable UV sensitivities but that the virion or V antigen is much more UV sensitive. The UV survival of SV40 functions in normal cells and cells from patients with xeroderma pigmentosum (XP) have been compared. In this disease, cells lack the ability to repair UV damage to cellular DNA. The results show that normal cells have the ability to repair UV damaged virus while XP cells do not.

A large scale screening program is underway to test human fibroblast cells from patients with a variety of diseases associated with a high incidence of tumors for their susceptibility to transformation by SV40.

Serological studies in American Burkitt's patients indicate that their antibody levels to EBV are much less than African patients with Burkitt's lymphoma. The titers in American Burkitt's patients were linked to prognosis, however, with patients having low levels of EBV antibody doing poorly. The higher immunoglobulin levels and the high incidence of EBV in normal Africans compared to normal Americans suggest that the differences in EBV titers may be related to factors other than etiology.

Herpes saimiri is a DNA containing virus indigenous to the squirrel monkey. Recent studies have shown that this virus will induce acute lymphocytic leukemias as well as lymphomas and reticulum cell sarcomas in owl monkeys and marmosets. This is the first demonstration that a virus produces leukemia and lymphomas in primates and may serve as an important model for human leukemia and lymphoma.

A very sensitive assay for the serum factor which induces cellular DNA synthesis in mouse cells in much the same way that the tumor viruses induce cellular DNA synthesis has been developed using 3T3 and Balb/3T3 cells. The characterization of the serum protein involved in the initiation of cell division will be important in understanding the normal mechanism by which cell division is controlled and how tumor viruses are able to overcome this control.

Other Activities of the Branch

During this reporting period, senior investigators of the Branch published a total of 74 papers which covered various aspects of viral oncology.

Members of the Branch presented by invitation over 50 lectures to research groups in this country and abroad, and over 30 abstracts were presented at various scientific meetings. The Branch entertained approximately 70 visitors for discussions in various aspects of viral oncology; this included representatives from every major research institution in this country and from 9 foreign countries. The Branch also provided training for periods up to six months in a variety of experimental procedures to outside visitors. This training consisted of orientation in immunological, biological and biochemical procedures as well as virus handling techniques.

Many of the investigations described in the Viral Oncology portion of this report depend on the availability of clinical and laboratory materials of optimal purity, viability, potency, etc. Indeed, comparative studies in an integrated program of international scope, as encompassed in the SVCP, can make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures and test animals are available.

The Resources and Logistics Activity provides viruses and antisera, human tissues, special laboratory animals (including infectious leukosis virus-free eggs), all produced, characterized, stored and distributed under various contract operations. Diagnostic services for the detection of murine, non-human primate, and cat viruses, and the viral reagents for these tests, are also provided.

An annually revised catalog lists and describes all resources available through the Resources and Logistics Activity. Usually the information on each item includes origin, processing procedure, degree of purity (freedom of contamination), infectious titer, or other biological activity. Human materials, including sera, tumors, cell outgrowths from tumors and normal tissues, are obtained for distribution to qualified investigators. A central inventory of information on each specimen is being developed which should greatly facilitate matching requests for human resource material with specimens, regardless of the geographic location of the laboratory in which it is stored.

About one and one-half years ago, a nationwide effort was initiated to obtain cancerous cats (primarily leukemias and sarcomas) from cat breeders and owners. Research laboratories local specimen supply has been supplemented by the nationwide contacts for materials and cancerous pure-bred cats with pedigrees. Letters are being sent to cat owners soliciting cats with veterinarian diagnosed cancers. The procurement effort not only obtains valuable research material but reassures the cat owners and other concerned citizens of NCI's active program for the evaluation of the unlikely hazard of this disease to human health.

In addition, several of the senior investigators within the Branch spent a portion of their time in support of the Special Virus Cancer Program. The members serve as Chairmen, Vice Chairmen, Work Group members and Secretaries of the major segments of the Program. They provide scientific guidance as Project and Assistant Project Officers on research contracts supported by the Special Virus Cancer Program.

The activities of the SVCP and the direction of the internal research program of the Branch are aimed at the common goal of the determination of the viral etiology of human cancer. It is apparent that the efforts of the Branch members have played a significant role in the progress of the SVCP to date. The broad scientific perspective developed by these investigators in their SVCP activities has also contributed significantly to the direction of the Branch program for the attainment of research goals.

The effective functioning of senior personnel in dual capacities, i.e., in-house research and program administration requires a delicate balance of effort. It must be realized through constant monitoring, that such a balance does exist and over-emphasis in either direction would be to the detriment of both programs. It has become clearer during the past year that an understanding of the suspected relationship between tumor viruses and human neoplasia requires an interaction between, among others, highly skilled molecular biologists, epidemiologists, cell biologists and physicians, along with sound and constructive administrative support; the answers will come from no one discipline alone.

Serial No. NCI-4821
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Genetic and Environmental Factors in Susceptibility to Tumor Viruses

Previous Serial Numbers: 4904, 4906, 4919

Principal Investigator: Dr. George J. Todaro

Other Investigators: Dr. Stuart A. Aaronson

Cooperating Units: Inside NIH
Dr. Paul Levine, VLLB, NCI
Dr. Wade Parks, VCB, NCI
Epidemiology Branch, NCI

Outside NIH
Naval Biological Laboratory, Oakland, California

Man Years:

Total:	4.00
Professional:	1.00
Other:	3.00

Project Description

Objectives:

1. To develop a rapid assay for cellular sensitivity to viral transformation suitable for screening human populations.

2. Determination of the reason for the difference between normal and susceptible human cell strains with regard to transformation by SV40 virus.

3. To test if the difference in susceptibility found with the DNA viruses can also be shown for oncogenic RNA viruses.

4. To utilize susceptible human cells and the most optimal conditions to search for other transforming viruses for human cells.

5. To select viral mutants that have lost their ability to transform cells.

6. To study the ability of RNA and DNA from tumor viruses to transform human cells.

7. To study how agents such as radiation and chemical carcinogens act to facilitate transformation.

Methods Employed:

Standard techniques of tissue culture, virus isolation and virus antigen detection are being used.

Major Findings:

1. A markedly increased susceptibility to transformation has been found with cells from eight individuals with Fanconi's anemia and five individuals with Down's syndrome. Both of these genetic diseases are associated with a high incidence of spontaneous tumors. In addition, cell strains from three out of five members of a "sarcoma prone" family exhibited very high susceptibility to transformation. The quantitative assay which is used to measure susceptibility to transformation determines the fraction of cells which can form transformed clones after exposure to the virus. With normal human diploid cells at a multiplicity of infection of 1,000, this fraction is .02 - .05 percent. With the cells from the individuals with Fanconi's anemia and the sarcoma prone family, the transformation frequency is 10 to 100 times higher; with Down's syndrome it is 3 to 5 times higher.

2. Susceptible cells by the SV40 transformation test also appear to be more susceptible to transformation by murine sarcoma viruses (Kirsten and Moloney strains).

3. About 5 percent of the normal population shows unusual susceptibility to SV40 transformation. These individuals may also then be at increased risks of developing cancer.

4. Over 700 human fibroblast strains have been tested for their SV40 transformation susceptibility. These include cells from normal persons of all ages, cells from certain partially inbred human populations, cells from relatives of tumor patients and cells from various "high risk" clinical conditions. The results will be tabulated and correlated within the next few months.

Significance to Biomedical Research and the Program of the Institute:

1. These studies suggest a possible simple test for "cancer proneness" in man. They may allow us to recognize a "high risk" group for cancer in the human population.

2. The most susceptible cells would appear to offer the best possibility for use as test cells in the search for hitherto undetected tumor viruses of man.

Proposed Course:

The objective of the above study is to see if a simple *in vitro* test can be used to measure a person's "risk" of developing cancer. The testing of additional populations will make clear how far the initial observations can be extended and whether this approach will be clinically useful. The factor(s) in cells that are responsible both for susceptibility and resistance will be studied with the goal of characterizing these factors in biochemical terms.

Honors and Awards:

Ten Outstanding Young Men of America, 1970, named by United States Jaycees

Publications:

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Parks, W.P., Scolnick, E.M., Todaro, G.J. and Aaronson, S.A.: RNA-dependent DNA polymerase in primate syncytium-forming ("foamy") viruses. Nature 229: 257-260, 1971.

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Serial No. NCI-4822
1. Viral Leukemia and Lymphoma Branch
2. OASDVO, Etiology
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) The role of RNA viruses in human leukemia and breast cancer
(B) EBV studies in humans and non-human primates

Previous Serial Number: NCI 4811

Principal Investigator: Dr. Paul H. Levine

Other Investigators: None

Cooperating Units: Inside NIH:
Dr. Ernest Plata, VLLB, NCI
Dr. Deward Waggoner, VLLB, NCI
Dr. Ronald Herberman, GL&C, NCI
Dr. Costan W. Berard, LP, NCI
Dr. Gregory T. O'Conor, LP, NCI
Members of the Medicine, Viral Biology, Immunology - GL&C, and Epidemiology Branches, NCI

Outside NIH:
Dr. L. Dabich, Simpson Memorial Inst., Ann Arbor, Mich.
Dr. Charles Rickard, Cornell University, Ithaca, N.Y.
Bionetics Research Laboratory, Bethesda, Md.
Hospital for Sick Children, Toronto, Canada
University of Colorado Medical Center, Denver, Colo.
Pfizer and Company, Maywood, New Jersey

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

Project A.

1. To pursue preliminary studies indicating that there is an antigen in human leukemia cells which cross-reacts with murine and/or feline leukemia viruses.

2. Using identical twins, to identify a leukemia specific antigen with humoral and cellular-mediated immunological assays. To determine whether this antigen can be detected by family members and controls. To evaluate techniques in this system which might be more sensitive and practical in providing evidence as to the etiology and control of human leukemia.

3. To use existing seriological techniques which are sensitive and reliable in the detection of animal leukemia and breast tumor viruses and determine epidemiologically whether there is evidence for horizontal and/or vertical transmission of viruses with similar antigens in humans.

Project B.
1. To develop seroepidemiological studies using viral capsid antibody and antibody to the early antigen to determine whether EBV is etiologically related to human lymphoma.

2. To investigate the role of cellular immunity to EBV as a possible controlling factor of antibodies to the viral capsid antibody.

3. To develop a comparable system for studying humoral and cellular immunity to EBV in Rhesus monkeys and, using immunosuppression, attempt to induce lymphomas using EBV.

Methods Employed:
Project A.
1. Serum samples were obtained from patients with a variety of diseases and normal controls. The sera were tested against Rauscher leukemia virus by passive hemagglutination, complement fixation, immunodiffusion, immunofluorescence, and neutralization. Antibodies against cat leukemia virus were detected by passive hemagglutination, immunodiffusion, and neutralization. Antibodies to mammary tumor virus were detected by passive hemagglutination and immunofluorescence. A human cell line derived from a breast cancer patient which has been identified as containing "B" or "C" type particles by electron microscopy was used as a source of antigen using complement fixation.

2. Eight patients with acute leukemia and their identical twins were evaluated by a variety of techniques. Lymphocyte cytotoxicity, mixed lymphocyte culture and skin testing were used to determine whether cellular immunity factors could identify a tumor specific antigen, and whether there was evidence that this antigen caused immune reactivity in family members and controls. Cells from the patient and his identical twin were used as sources of antigens directly and materials were also placed in tissue culture. Skin fibroblasts from family members were tested to determine whether any genetic factors could be associated with acute leukemia. Electron microscopy and immunofluorescence, using antisera against Rauscher and feline leukemia virus, would be utilized to determine whether any of the antigens detected by cellular immunity tests were related to any known animal leukemia viruses.

Project B.

1. Blood samples were collected from a variety of lymphoma patients and normal controls. Cell lines known to contain EB virus, cell lines suspected of containing the EBV genome, and cell lines without evidence of EBV were used as a source of antigen in these studies. Sera were tested for antibody to the viral capsid antigen and the early antigen by immunoflourescence. Lymphocyte cytotoxicity was employed as a test of cellular immunity.

2. Rhesus monkeys were inoculated with large doses of infective HRlK virus. Serum samples were collected for antibody determination and careful clinical monitoring was performed to look for clinical evidence of disease. Heavy doses of chemotherapy were administered to some of the monkeys to determine whether the response to virus could be altered by drugs, either immunologically or clinically. The continued investigation on the monitoring of the immunosuppressive state was developed with vaccinia virus being employed instead of Newcastle's disease virus, and dose response testing with DNCB was pursued.

Major Findings:

1. Serological reactivity against Rauscher leukemia virus, feline leukemia virus, mammary tumor virus and the Belev cell line were identified in a variety of cancer patients and controls. The clearest evidence for disease specificity was provided by the studies in the Belev cell line, which demonstrated antibody in 40% of breast cancer patients and less than 10% in matched controls. Patients with various forms of cancer undergoing similar surgical and chemotherapeutic procedures possessed antibodies within normal range. A higher reactivity against the animal leukemia virus was found in certain groups working in viral oncology laboratories, but further studies need to be performed to determine whether this is reactivity to virus or to other animal antigens.

2. A leukemia associated antigen was identified in 6 of the 8 pairs of identical twins using lymphocyte cytotoxicity test. Family members and unrelated donors reacted against the patient's cells and not the normal twin indicating that the leukemia antigen could be provoking an immune response in other individuals, therefore supporting the viral etiology theory. Mixed leukocyte cultures and immunofluorescence did not determine any different antigens in the leukemia patient as opposed to the identical twin, but the majority of the studies were performed when the patients were in remission. In other ongoing studies, both immunofluorescence and MLC have detected leukemia associated antigens and it is suspected that when patients with active disease are studied these tests may also be positive. However, the types of antigens detected by each of these tests are different so that a complete correlation is not expected.

3. Serological studies in American Burkitt's patients indicate that their antibody levels to EBV are much less than African patients with Burkitt's lymphoma. The titers in American Burkitt's patients were linked to

prognosis, however,with patients having low levels of EBV antibody doing poorly. The higher immunoglobulin levels and incidence of EBV in normal Africans compared to normal Americans suggest that the differences in EBV titers may be related to factors other than etiology. Serial studies in patients with Hodgkin's disease and chronic lymphocytic leukemia suggested entirely different patterns. Patients with CLL, who had elevated EBV antibodies, developed them prior to any major disease, while Hodgkin's patients with high EBV titers, in contrast to the American Burkitt's patients, have a poorer prognosis than those with low titers. The studies to date indicate that EBV has a different pattern in different lymphomas and that the information obtained in one lymphoma cannot be extrapolated to another lymphoma. The importance of prospective studies and careful histological examination was emphasized.

4. Seven new cases of concurrent infectious mononucleosis and acute leukemia were identified and EBV titers were performed. Serological evidence against the common etiology for acute leukemia and infectious mononucleosis was found. Also, two patients with positive antibody levels to EBV 3 months prior to the onset of infectious mononucleosis and one patient with recurrent documented infectious mononucleosis supported previous studies indicating that infectious mononucleosis is not necessarily the result of a primary exposure to EBV.

Significance to Bio-Medical Research and the Program of the Institute

1. The studies in acute leukemia, both the serological studies and the twin studies, indicate that humans may produce an immune response to leukemia associated antigens. If this work is supported by further studies, the use of vaccines in the prevention and treatment of acute leukemia could be a reality. These vaccines, both preventive and therapeutic, can be evaluated in the absence of a clearly isolated human leukemia virus. In addition, the study supports the belief that human leukemia is a virus induced disease.

2. The seroepidemiological studies in breast cancer also indicate that there is a common breast cancer antigen. The identification of common tumor antigens support an oncogenic virus hypothesis. Since other studies have given evidence for common breast cancer antigens using skin testing as a method of cellular immunity, it is now possible to activate a comprehensive clinical program in breast cancer using well established techniques to identify whether these common antigens that are being detected are indeed viral and whether the immune response that breast cancer patients are mounting can be applied to the identification, prevention, and/or treatment of breast cancer in humans.

3. Studies in EBV have now linked the virus to a number of diseases: post nasal carcinoma; Burkitt's lymphoma; Hodgkin's disease; chronic lymphocytic leukemia; sarcoidosis, and several others. The evidence for etiology, however, is not conclusive. The studies described above indicate that each

disease has a different pattern of EBV related immunity, and that information obtained from one disease, therefore, cannot be applied clearly to other lymphomas. The advantages of a well designed epidemiological study in the United States to determine whether a pattern of antibody can be correlated with the incidence of certain lymphomas can now be based on laboratory evidence so that any results are more likely to be conclusive.

4. The studies of Rhesus monkeys have suggested that a biological effect can be obtained by infective EBV. The lack of availability of sufficient high titered virus has slowed down the progress of this study. The development of techniques following cellular and humoral immunity in Rhesus monkeys, however, has provided a basis for a careful systematic study using immunosuppression and/or immunostimulation in conjunction with EBV to increase the chance that this virus can be shown to be oncogenic in a species very similar to man.

Proposed Course:

Project A.

1. Seroepidemiological studies in cluster and non-cluster areas of acute leukemia will be attempted to determine whether there is a difference in cellular immunity to leukemia-associated antigens in the two areas. A positive correlation will clearly support the environmental hypothesis whereas a negative study would be strong evidence against it.

2. The specificity of the seriological reactions in people against Rauscher leukemia virus, cat leukemia virus, and mammary tumor virus will be pursued by obtaining more sera from positive individuals which can be characterized more fully and their specificity determined.

3. The study of identical twins will continue in an attempt to more carefully define the specificity of the leukemia associated antigens and the immune response of humans to these antigens. New tests will be applied to the study of twins as they are developed, since these families are ideal for the study of genetic and immunological factors associated with etiology of acute leukemia. In addition, twins with other forms of cancer will be sought and methodology successfully applied to the twin leukemia study will be applied to the other twin families as well.

Project B.

1. Prospective studies in patients at risk to lymphoma, in patients with infectious mononucleosis, and lymphoma patients will be attempted using serological and cellular immunity tests to EBV. An attempt will be made to correlate lymphoma specific antigens and EBV-related antigens to determine indirectly whether EBV is consistently associated with any lymphomas. Genetic aspects will be investigated since it is possible that a variety of diseases are the result of genetic response to the same virus rather than virological factors.

2. The studies in Rhesus monkeys will be pursued in collaboration with Dr. Gary Pearson and investigators at Bionetics Research Laboratories. Similar immunological systems will be established in human and monkeys so that if EBV is etiologically related in human lymphomas, the Rhesus monkey can be manipulated to develop those factors associated with human lymphomas and the oncogenicity of EBV can more readily be demonstrated. Vaccine trials to demonstrate potency of EBV-related vaccines could be developed in these monkey systems.

Honors and Awards:

1. Elected as Fellow of the American College of Physicians.
2. Selected for membership in Immunotherapy Subcommittee of Clinical Research Committee, NCI.

Presentations:

1. "Current Concepts of Viral Oncogenesis" at the Brain and Bone Symposium, Denver, Colorado, January 21 & 22, 1971.
2. "The Current Status of EBV in Human Lymphoma", Georgetown Medical Center, April 22, 1971.
3. "Leukemia in Identical Twins: New Implications for Viral Oncology", American College of Physicians, Denver, Colorado, March 31, 1971.
4. "Immunologic Studies with a Cell Line from a Patient with Breast Cancer", American Society for Microbiologists, Minneapolis, Minnesota, May 3, 1971. (coauthor)
5. "The relationship of Epstein-Barr Virus Antibodies to Disease State in Hodgkin's Disease, Chronic Lymphocytic Leukemia and American Burkitt's Lymphoma", Symposium on Oncogenesis and Herpes-Type Viruses, Cambridge, England, June 22, 1971.

Publications:

Levine, P. H., Merrill, D. A., Kohler, P. F., and Claman, H. N.: Changes in serum immunoglobulin and complement levels following renal hemotransplantation. Transplantation 10: 141-154, 1970.

Levine, P. H., Ablashi, D. V., Berard, C. W., Carbone, P. P., Waggoner, D.E., and Malan, L.: Elevated antibody titers to Epstein-Barr virus in Hodgkin's disease. Cancer 27: 416-421, 1971.

Stevens, D. A., Levine, P. H., Lee, S. K., Sonley, M. J. and Waggoner, D. E.: Concurrent infectious mononucleosis and acute leukemia. American Journal of Medicine 50: 208-217, 1971.

Levine, P. H.: Wanted: Case Reports of Burkitt's Lymphoma in the U. S.. Medical Times 99: 124-126, 1971.

Serial No. NCI-4823
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A. Studies with murine sarcoma virus (MSV)-transformed mouse 3T3 cells which are negative for leukemia virus

B. The development of rapid in vitro assay techniques for murine leukemia viruses.

C. Comparative effects of DEAE-dextran on the oncogenicity in vivo and focus forming ability of MSV

D. Transformation of cat cells by a feline psuedotype of MSV in agar suspension cultures.

Previous Serial Number: NCI-4813

Principal Investigators: Dr. Robert H. Bassin
Dr. Peter J. Fischinger

Other Investigators: Dr. Adi Gazdar
Dr. Daniel Haapala, VLLB, NCI
Dr. Michael J. Kramer, VBB, NCI
Dr. Kyu M. Lee, VLLB, NCI
Dr. Shigeko Nomura, VLLB, NCI
Dr. Paul T. Peebles, VLLB, NCI
Dr. Leo A. Phillips, VLLB, NCI

Cooperating Units: Inside NIH
Staff, VLLB, NCI
Staff, Electron Microscopy Section, VBB, NCI

Outside NIH
None

Man Years:

Total:	4.0
Professional:	2.0
Others:	2.0

Serial No. NCI-4823

Project Description

Objectives:

A. To characterize clonal isolates of 3T3 cells transformed by murine sarcoma virus in the absence of replicating leukemia virus using techniques of biochemistry, immunology, virology, and electron microscopy.

B. To develop rapid and sensitive *in vitro* assay techniques for murine leukemia viruses based on the induction of lytic foci in mouse cells previously transformed by murine sarcoma virus.

C. To determine whether DEAE-dextran exerts an enhancing effect on tumor induction by murine sarcoma virus comparable to its enhancement of MSV focus formation *in vitro*.

D. To develop a system for measuring transformation of cat cells by a feline pseudotype of MSV independent of virus replication using agar suspension cultures.

Methods Employed:

Standard cell culture procedures were employed including the cloning of cells in microplates, transformation, and focus assays in a variety of cell types both in monolayers and in agar suspension cultures. Sucrose density gradient analysis of selected culture fluids was carried out following incorporation of H^3-uridine. Standard techniques of electron microscopy were employed for detection of virus-like particles.

Major Findings:

A. The isolation of S+L- 3T3 cells which have been transformed by MSV but do not release infectious leukemia or sarcoma virus has been described previously. Two S+L- lines were selected for further study and were cloned by 2 successive isolations of individual colonies grown in Falcon microplates. Examination of both S+L- clones in the electron microscope revealed the presence of small numbers of virus-like particles conforming in size and morphology to murine leukemia and sarcoma viruses. Incorporation of H^3-uridine into material banding at a density of 1.16 g/ml in sucrose gradients also confirmed the release of virus-like particles.

Repeated attempts to demonstrate infectivity or transforming activity associated with S+L- culture fluids and cell lysates were negative. Various target cells and culture conditions were employed, including addition of helper viruses and DEAE-dextran to assay cells, cosedementation murine and feline helper viruses, and infection in the presence of UV-inactivated Sendai virus. Concentrated S+L- fluids were also negative. The S+L- particles seem to represent a "defective" form of sarcoma virion, possibly analogous to RSV (0).

B. The isolation of cloned S+L- cell lines has made possible the development of a rapid and reproducible assay technique for murine leukemia viruses. Infection of cloned S+L- cells with suitably diluted preparations of MuLV is followed by the formation of lytic foci by a mechanism as yet unknown. The sensitivity of this technique is comparable to the XC plaque test on other cumbersome techniques such as the induction of viral interference. Assays of culture fluids obtained from MuLV-superinfected S+L- plates indicated that leukemia virus replication is required for the induction of lytic foci as well as for the rescue of MSV from S+L- cells. A micro-assay based on the response of S+L- cells to infection with MuLV has been developed using Falcon microplates. This technique may be of use for cloning leukemia viruses as well as for analyzing the products of single virus-cell interactions.

C. DEAE-dextran exhibited a marked, but variable, degree of enhancement of focus formation by different stocks of MSV. The degree of enhancement seemed to depend on the particular batch of medium in which a particular virus pool was suspended. Treatment of mice with DEAE-dextran either systemically or locally also resulted in a significant enhancement of the oncogenicity of MSV as measured by several different parameters.

D. The Crandall cat cell line (CCC), a continuous line of feline kidney cells, is susceptible to virus replication and cell transformation by MSV(FeLV). A detailed quantitative analysis of focus formation by defective MSV(FeLV) in the presence or absence of added FeLV "helper" virus has shown the CCC line to be nearly equal to feline embryo cells in its sensitivity to these agents. Since CCC cells are highly contact inhibited, they are ideally suited to a study of transformation in agar suspension cultures following infection with MSV(FeLV). Initial experiments indicate that infection of CCC cells does induce the formation of colonies of transformed cells in agar suspension cultures. We are currently attempting to isolate S+L- cat cells by this procedure.

Significance to Biomedical Research and the Program of the Institute:

A. An analysis of the "defectiveness" of RNA sarcoma viruses is of great value in determining how these agents interact with leukemia "helper" viruses and how they may be detected in the absence of virus infectivity. This type of information would be of critical importance in assigning a viral etiology to "spontaneous" tumors from which no infectious virus can be recovered. In addition, if the specific viral genes (and their products) responsible for cell transformation by sarcoma viruses can be identified, a great deal of information can be gained regarding the mechanism of cell transformation. A comparative study of different cell lines transformed by MSV may contribute to this knowledge.

B. Rapid *in vitro* assay techniques for murine leukemia viruses are of great value in the detection and quantitation of these agents and their antisera. The replication of leukemia viruses under a variety of conditions has been quantitated and studies such as the effects of inhibitors on MuLV replicates have been made feasible.

C. The use of DEAE-dextran to enhance the oncogenicity of MSV *in vivo* may be useful in the detection and quantitation of other oncogenic RNA viruses, particularly when studies involve the induction of tumors in heterologous species.

D. Cell transformation in agar suspension cultures has proven to be a useful method for isolating mouse cells infected with MSV in the absence of replicating leukemia virus. These cells, in turn, can be used to develop sensitive assay techniques for murine leukemia viruses. Similar results with cat and, eventually, human cells would have obvious advantages in these systems.

Proposed Course:

A. A detailed analysis of the particles released by S+L- cells is underway. It is anticipated that a study of S+L- cells before and after superinfection with MuLV will be of value in determining the nature of MSV "defectiveness" and the role of MuLV "helper" virus.

B. New assay techniques for MuLV are being employed in a variety of ways including a study of the kinetics of MuLV replication under various conditions, an analysis of the antigenic relationships of various leukemia viruses, and a search for neutralizing antibodies to MuLV in man.

C. No further experiments in this area are planned.

D. An effort will be made to isolate CCC cells infected by MSV(FeLV) alone in an effort to isolate S+L- cat cells. This work will also be applied to human cell lines.

Honors and Awards:

Dr. Bassin presented a lecture entitled "Cell Culture Approaches to the Virus Cancer Problem" to the Veteran's Administration Hospital, Washington, D.C. on November 12, 1970.

On March 19, 1971, Dr. Fischinger presented a lecture to the Institute of Medical Microbiology, University of Copenhagen.

Dr. Fischinger was awarded a foreign assignment for study at the Max-Planck Institut fur Virusforschung, Tubingen, Germany, under the sponsorship of Dr. W. Schafer for a period of one year beginning October 1, 1970.

Publications:

Bassin, R. H., Tuttle, N., and Fischinger, P. J.: Isolation of murine sarcoma virus-transformed mouse cells which are negative for leukemia virus from agar suspension cultures. Int. J. Cancer 6: 95-107, 1970

Bassin, R. H., Tuttle, N., and Fischinger, P. J.: A rapid cell culture assay technique for murine leukaemia viruses. Nature 229: 564-567, 1971

Turner, W., Ebert, P. S., Bassin, R. H., Spahn, C., and Chirigos, M. A.: Potentiation of murine sarcoma virus (Harvey) oncogenicity in lactic dehydrogenase-elevating virus-infected mice. Proc. Soc. Exptl Biol. Med. In press.

Chesterman, F. C., Harvey, J. J., Branca, M., Phillips, D. E. H., Hallowes, R. C., and Bassin, R. H.: Tumors and other lesions induced by murine sarcoma viruses. In Homburgar, F. (Ed.): Pathology of the Syrian Hamster. New York, S. Karger. In press

Gazdar, A. F., Russell, E., and Bassin, R. H.: In vivo enhancement of a murine sarcoma virus by diethylaminoethyl-dextran. Proc. Soc. Exptl. Biol. Med. In press

Fischinger, P. J. and Moore, C. O.: Quantitation of human cell transformation by a modified sarcoma virus. J. Gen. Virol. In press

Serial No. NCI-4824
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies with RNA oncogenic viruses

Previous Serial Number: NCI-4814

Principal Investigator: Dr. Adi F. Gazdar

Other Investigators: Dr. Paul Peebles
Dr. John Easton
Dr. Robert H. Bassin
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Dr. Padman Sarma, VCB, NCI
Dr. Norman Talal, LAR, NIAMD
Dr. Alfred Steinberg, LAR, NIAMD
Dr. Sam Baron, LBV, NIAID
Dr. Harish Chopra, VB, NCI

Outside NIH
Dr. Gerald Spahn, Microbiological Associates
Walkersville, Maryland

Man Years:

Total:	5.0
Professional:	1.0
Other:	4.0

Project Description

Objectives:

1. To characterize the changes in the properties of a murine sarcoma virus after hamster passage.

2. To investigate the relationship between certain viruses, autoimmune disease and neoplasia in New Zealand mice.

3. To investigate the relationship between the interferon system, oncogenic viruses, and susceptibility to oncogenesis.

4. To investigate the mode of action of polyribonucleotides in murine sarcoma and leukemia systems.

5. To develop rapid *in vivo* assay systems for the scrapie agent, by investigating its interference with oncogenic viruses.

Methods Employed:

1. Immunologic tests include complement fixation, hemagglutination, hemagglutination inhibition, and fluorescent antibody tests.

2. The progress of autoimmune disease is followed by radio-immunoassay tests for antibodies to RNA and DNA.

3. Interferon assays are performed using an inhibition of hemagglutinin yield method.

4. The effects of polyribonucleotides on the interferon system, immune system, tumor development, and virus yield are studied.

5. The effects of scrapie on the pathogenicity and yield of oncogenic viruses and arboviruses are studied.

Major Findings :

1. A potent sarcoma-inducing virus was recently isolated after *in vivo* passage of a spontaneous tumor arising in an NZB/NZW F_1 mouse. It is a type C virus, contains the gs antigen of the murine leukemia virus group, causes discrete focal lesions in mouse cell cultures, and is associated with a non-cytopathogenic type C virus. It rapidly induces local tumors in mice, hamsters, rats, and mastomys. Hamster tumors and cell culture lines derived from the tumors produce large amounts of a type C virus which contains the murine gs antigen but appears to be non-infectious. Recent findings suggest that the hamster-derived virus is the sarcoma virus synthesized in the apparent absence of the associated "helper virus" present in mouse-derived virus stocks.

2. The New Zealand (NZ) strains of mice spontaneously develop autoimmune disease and are a useful model for human systemic lupus erythematosus. A type C virus is present in many of the tissues of NZ mice, and has been associated with immune-complex glomerulonephritis as well as the high incidence of lymphoma in NZ strains. Introduction of murine leukemia viruses (MuLV) into NZB/NZW F_1 mice at an early age enhances the appearance of antibodies to RNA. Repeated injections of polyribonucleotides have a similar effect. Mice made tolerant to Poly I.C show a delay in the appearance of spontaneous and MuLV induced antibodies to RNA. The data indicates that the mechanism of production of antibodies to RNA following inoculation of MuLV is similar to that operative in the natural state.

3. The effect of the murine leukemia-sarcoma viruses on the interferon response has been investigated. Eleven virus strains have been tested in four mouse strains. Virus infection fails to produce detectable serum interferon levels. However, starting at 24 hours, a marked depression of the NDV-induced interferon response is present. The effect persists for at least four weeks. The response to Poly I.C is only moderately depressed after type C virus infection. As NDV is believed to release interferon mainly from lymphocytes and Poly I.C from a variety of cell types, the differential depression of responses to interferon inducers may reflect a virus effect on lymphoid cells. The depression of interferon response is not related to differences in mouse strain susceptibility to oncogenic viruses, and is also produced by non-pathogenic virus strains. Thus depression of interferon responses is probably not related to oncogenesis.

4. Poly I.C, a synthetic polyribonucleotide, is a potent inducer of interferon. As treatment with Poly I.C can suppress oncogenesis by the Moloney sarcoma virus (M-MSV) this system was studied in detail. Only repeated injections of highly toxic doses of Poly I.C can suppress tumor formation at terminal virus dilutions. Tumor suppression is independent of circulating interferon production, and is accompanied by persistence of the viral genome. Suppression of M-MSV tumor induction is only seen in two of seven mouse strains tested. In addition, a single inoculation of Poly I.C greatly enhances M-MSV tumor induction in certain strains of mice associated with autoimmune phenomena.

5. Scrapie is a sheep disease associated with a filterable agent. Inoculation of the agent into sheep, goats, and mice results in the development of degenerative brain lesions after a long latent period. Preliminary data indicates that the scrapie agent contains no DNA or RNA, and may belong to a completely new class of infectious agent. Work on this highly interesting agent is handicapped by lack of a rapid assay system. As the initial replication of the scrapie agent occurs in the spleen and lymphoid tissues, its effects on the replication and pathogenicity of various oncogenic and arboviruses are being investigated. Preliminary data indicates that the scrapie agent interferes with certain other infectious agents.

Proposed Course:

1. The physical and antigenic properties of the sarcoma virus synthesized in the presence and absence of associated "helper virus" will be compared and contrasted.

2. The effect of several DNA and RNA oncogenic and non-oncogenic viruses on autoimmune disease in NZ mice will be studied.

3. The effect of oncogenic RNA viruses on the interferon responses in vivo and in vitro will be further characterized.

4. The apparently paradoxical enhancement by Poly I.C of M-MSV tumor induction in certain mouse strains will be studied and contrasted to its tumor suppressive action in other strains.

5. Based on viral interference, attempts will be made to develop relatively rapid in vivo assays for the scrapie agent.

Significance to Biomedical Research and the Program of the Institute:

Investigations on the nature and mode of action of certain animal type C oncogenic viruses, especially as they effect species other than that of origin will contribute to a more complete understanding of certain human cancers. Indeed, prevention or control of cancer is dependent upon the elucidation of mechanisms by which viruses manipulate species barriers and further definition of host response to oncogenic agents. Characterizing the properties of "defective" oncogenic viruses will aid in the search for putative human sarcoma viruses.

Honors and Awards:

Dr. Gazdar presented a paper entitled "The effects of DEAE-D on tumor induction by a murine sarcoma virus" to the American Association of Cancer Research.

Dr. Gazdar presented a paper entitled "Isolation and characterization of a new strain of murine sarcoma virus" to the American Society for Microbiology.

Dr. Gazdar presented a seminar entitled "The effects of Poly I.C on the Moloney sarcoma virus" to the Laboratory of Biology of Viruses, NIAID.

Publications:

Gazdar, A.: The response of NZW x NZB F_1 hybrid mice to a murine sarcoma virus. Int. Arch. Allergy and Immunol. 38: 509-513, 1970

Gazdar, A. and Dammin, G.: Neural degeneration and regeneration in human renal transplants. New Eng. J. Med. 283: 222-224, 1970

Levy, H., Adamson, R., Carbone P., DeVita, V., Gazdar, A., Rhim, J., Weinstein, A., and Riley, F.: Studies on the antitumor action of Poly I: Poly C. Beers, R. and Braun, W. (Eds.): Biological Effects of Polynucleotides. New York, Springer-Verlag, 1970, in press.

Weinstein, A., Gazdar, A., Sims, H., and Levy, H.: Poly I: Poly C: antitumor effect is independent of circulating interferon induction. Nature. In press.

Rabstein, L., Gazdar, A., Chopra, H., and Abelson, A.: The early morphological changes associated with a murine non-thymic lymphoma virus. J. National Cancer Inst. In press.

Gazdar, A., Beitzel, W., and Talal, N.: Age related responses of New Zealand mice to a murine sarcoma virus. Clin. and Exp. Immunol. In press

Gazdar, A., Russell, E., and Bassin, R.: In vivo enhancement of a murine sarcoma virus by diethylaminoethyl-dextran. Proc. Soc. Exp. Biol. Med. In press.

Talal, N., Steinberg, A., Jacobs, M., Chused, T., and Gazdar, A.: Immune cell cooperation, viruses, and antibodies to nucleic acids in New Zealand mice. J. Exp. Med. (Supp.). In press.

Serial No. NCI-4825
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Enzymes and nucleic acids associated with oncorna virions: A comparative study

Previous Serial Number: NCI-4837

Principal Investigator: Dr. Daniel K. Haapala

Other Investigators:
Dr. Robert H. Bassin, VLLB, NCI
Dr. Peter J. Fischinger, VLLB, NCI
Dr. Shigeko Nomura, VLLB, NCI
Dr. Paul T. Peebles, VLLB, NCI
Dr. Leo A. Phillips, VLLB, NCI

Cooperating Units: Inside NIH:
Electron Microscopy Section, VBB, NCI

Outside NIH:
None

Man Years:

Total	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To determine the occurrences and specificity of the RNA-dependent DNA polymerase associated with RNA tumor viruses.

Methods Employed:

Standard biochemical and biophysical methods were used. Hydroxyapatite column chromatography, combined with radioisotopic labelling was employed for discriminating single from double stranded forms of nucleic acids.

Major Findings:

Hydroxyapatite column chromatography was used to characterize the DNA synthesized by the Gardner feline leukemia-sarcoma complex *in vitro*. Most of the product was found to exist as a DNA-RNA hybrid, indicating that the polymerase is capable of utilizing RNA as a template for the synthesis of a specific DNA product. Reassociation experiments showed that while most of the DNA produced by Gardner virus enzyme could be reassociated with Gardner virus RNA, little if any homology could be shown with RNA's from other sources, including that obtained from Rauscher leukemia virus.

In a separate study, S+L- mouse cells, which contain the MSV genome in the absence of replicating MSV and MuLV, were shown to release apparently noninfectious virions visible in the electron microscope and detectable by incorporation of tritiated uridine. Careful analysis of S+L- particles showed them to be deficient in polymerase activity, even with exogenous rAdT added as template. The significance of these findings is under investigation.

Significance to Biomedical Research and the Program of the Institute:

The RNA-dependent DNA polymerases associated with oncorna virions may represent a unique tool with which to detect, characterize, and possibly, inhibit neoplastic changes resulting from infection with these agents. It is important, therefore, to determine the properties of these enzymes, especially their occurrence and specificity as well as the structure of the resulting DNA products.

Proposed Course:

A molecular "classification" of RNA tumor viruses using cross hybridization among various viral RNA's and their retroscribed DNA's is in progress, as is an examination of the functions of polymerase in the viral growth cycle.

Honors and Awards:

Dr. Haapala was a discussant and invited speaker at a symposium entitled "Enzymes Associated with Oncogenic Viruses" given during the Fifth Joint Working Conference of the Special Virus Cancer Program.

Dr. Haapala was a participant in the NIH-INSERM Information Exchange Program and spent three months studying at the Institut du Cancerologie et I'Immunogenetique, Villejuif, France, under the sponsorship of Dr. G. Mathe.

Publications:

Haapala, D. K. and Falkow, S.: Physico-chemical studies on the resistance transfer factor RTF in *Proteus mirabilis*. *J. Bacteriol.* In press

Serial No. NCI-4827
1. Viral Leukemia & Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: To determine the antigenic composition of murine leukemia viruses defining the geographic sites of the antigen in the virus following separation of the viral components.

Previous Serial Number: Same

Principal Investigator: Dr. Vincent W. Hollis, Jr.

Other Investigators: Dr. Ernest J. Plata
Dr. Mary A. Fink

Cooperating Units: Inside NIH:
Dr. Stuart A. Aaronson, NCI
Dr. Edward M. Scolnick, NCI
Dr. George J. Todaro, NCI

Outside NIH
Dr. Kyu M. Lee, Cornell University
Dr. Norman Weliky, TRW Systems Group

Man Years:

Total:	2.75
Professional:	1.0
Other:	1.75

Project Description

Objectives:

The basic aims of this project are to isolate and purify the various antigenic components of the murine leukemia viruses, and later other viruses, (a) to determine the amino acid content and sequence of these antigenic components; (b) to test these antigenic components for their ability to cross react with other tumor viruses; and (c) to determine which of the purified fractions elicits the production of protective antibody when used as a vaccine.

Methods Employed:

Standard techniques of differential centrifugation, molecular-sieve (gel) chromatography, density gradient centrifugation, and disc-gel electrophoresis were used for the isolation and purification of viral components. In addition to the standard methods, we have used a newer procedure, isoelectric focusing, with some success. The identification of these antigens was achieved by use of the passive hemagglutination inhibition technique and by microimmunodiffusion (see Dr. Plata's report, NCI-4826).

Major Findings:

In collaboration with Dr. Plata, Rauscher leukemia virus, produced in JLSV-9 cells, was labelled with a radioactive amino acid. This virus was purified and used as a tag with unlabelled density purified virus from contract (PH43-66-98). The virus is then treated with Triton X-100, a non-ionic detergent, and a reducing agent. This procedure differs from previous tween 80-ether methods in the removal of the envelope and disruption of the virus is more complete. Following this treatment of the virus, isoelectric focusing was carried out in a 110 ml capacity column with 1% carrier ampholine solution in the pH range of 3 to 10 or 5 to 8 for various periods of time. A sucrose density gradient of 0 to 50% (W/V) was used to prevent convection. Three peaks containing the radioactive amino acid have been isolated with isoelectric pH's of 4.2, 6.10, and 6.25 respectively. The first peak appears to be similar to that isolated by Oroszlan, et. al. and identified as the envelope antigen. The second peak gave a line of identity with the group-specific antigen by immunodiffusion. The third peak has not been identified as yet.

Analysis of the virus by disc-gel electrophoresis indicates that there may be as many as six or seven viral proteins. We are presently attempting to obtain anti-sera to the fractions isolated to aid in identifying the viral antigens.

Experiments with Drs. Scolnick, Aaronson, and Todaro have used isoelectrofocusing in an attempt to purify the RNA dependent DNA polymerase enzyme. This work is still in progress.

Significance to Biomedical Research and the Program of the Institute:

The isolation, purification, and chemical characterization of the antigens of a murine leukemia virus contribute to the elucidation of the structure of individual viruses and will assist in determining definite interrelationships among other murine leukemia viruses. These and other procedures, when applied to the control or prevention of human leukemia may aid in the development of methods for immunological improvement of this disease; e.g., the use of sub-viral components in vaccines, which although antigenic are incapable of infecting the recipients of the vaccine.

Proposed Course:

Studies will continue in an effort to elucidate the chemistry of viruses that induce leukemia and solid neoplasms in the murine and feline species. More specifically, it is planned: (1) to continue the studies on the isolation, purification, and chemical identification of the antigens of a murine leukemia virus (Rauscher); (2) to attempt to define the time of onset viral protein synthesis, emphasizing the time of synthesis of the major antigens; (3) to begin studies concerned with the isolation, purification, and characterization of the antigens of murine mammary tumor, and feline leukemia viruses; and (5) to continue to collaborate with Drs. Scolnick, Aaronson, and Todaro in the isolation and purification of viral enzymes.

Honors and Awards:

None.

Publications:

Sibal, L. R., Hollis, V. W., Jr., and Fink, M. A.: Detection of antigenic subunits of a murine leukemia virus by passive hemagglutination inhibition. J. Immunology, in press.

Serial No. NCI-4828
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunological studies in animal and human tumors, to include characterization, diagnosis, therapy and prophylaxis of viral neoplasia

Previous Serial Number: NCI-4826

Principal Investigator: Dr. Ernest J. Plata

Other Investigators: Dr. Vincent W. Hollis, Jr.

Cooperating Units: Inside NIH

Dr. Mary A. Fink, NCI
Dr. Louis R. Sibal, NCI
Dr. Paul H. Levine, NCI
Dr. Harish C. Chopra, NCI
Dr. Deward Waggoner, NCI
Dr. George J. Burton, NCI

Outside NIH

Dr. Donald Mashburn, Washington Sanitarium and Hospital Takoma Park, Maryland
Dr. Marcus M. Mason, Mason Research Institute, Worcester, Massachusetts
Dr. Kyu M. Lee, Cornell University, Ithaca, New York
Dr. Paul Terasaki, University of California, Los Angeles, California
Dr. John Verna, Meloy Labs., Springfield, Virginia
Dr. Michael Viola, Howard University, Washington, D.C.
Dr. B. Y. Wat, Loma Linda University, Loma Linda, California

Man Years:

Total:	3.75
Professional:	1.00
Other	2.75

Serial No. NCI-4828

Project Description

Objectives:

1. To develop new, or to adapt current methods for the detection, identification, and characterization of viruses or specific antigens associated with neoplasia.

2. To study the humoral and cellular immune response of the host to its own tumor and to oncogenic viruses.

3. To explore applicable immunological models for improved diagnosis, improved therapy, or possible prophylaxis of cancer.

Methods Employed:

1. Detection of viruses or specific antigens associated with neoplasia.

A. Murine MTV Studies. A C_3H mouse mammary transplantable tumor rich in MTV virus and antigens was used as a model system. Tumors were fractionated by homogenization, sonication, salt treatment, differential centrifugation, and density gradients to yield whole MTV, viral antigens and soluble tumor antigens that could be clearly identified by passive hemagglutination, complement fixation, immuno-diffusion, and electron microscopy. Cell cultures of the same tumors were positive for viral antigens by fluorescent antibody microscopy, strongly on initial passages and diminishing consistently to extinction by passage 10.

B. Human Tumor Antigen Studies. The methodology developed with the Murine MTV system was applied to benign and malignant human breast tumor tissues. Phase one of this effort aims to collect and accumulate specific fractions from human tumors and cell cultures derived from human tumors to immunize rabbits. The antiserum will be examined for tumor specific antibodies. To date no cross-reactions have been detected between the antigens prepared from human tumors and those of murine MTV. Nine malignant and six benign tumors have been processed. The small size of human breast tumor specimens (2-4 grams each) and their relative scarcity make the accumulation of minimal quantities of selected antigens for study a slow undertaking. Two cell lines derived from human mammary adenocarcinoma have been grown beyond passage 10, out of 7 attempts. These cell lines and primary tumor imprints have been examined by electron microscopy and by indirect fluorescent antibody techniques to detect viral growth or tumor associated antigens. Certain tumor imprints and some normal tissues have shown positive fluorescence reactions with antibody against murine MTV. Other tumors, tissues, and cells of the Be-Lev established cell line have also been positive with some sera obtained from patients with breast cancer. Our preliminary data, to date, shows no direct corollation between the finding of antigens detectable by anti-MTV serum or by serum from breast tumor patients and human adenocarcinoma specimens. Questions concerning the specificity of such reactions continue to be examined.

C. Feline Tumor Studies. Cell lines were derived from a feline mammary adenocarcinoma and a feline fibrosarcoma. These cultures now in passages 3 and 7 respectively are being monitored by electron microscopy for viruses. Supernatant fluids were negative for FeLV or FeSV by passive hemagglutination to date. An autologous transplant of the original feline adenocarcinoma was successful. Transplants of the same tumor were made to kittens (less than 24 hours old) of similar genetic background. Preliminary observations may indicate successful transplantation. A transplantable breast tumor in cats, if successful, besides its intrinsic interest, may provide a working model for the human disease.

Serological methods were developed for the detection of feline leukemia virus antigens and antibodies. These included the passive hemagglutination and hemagglutination inhibition tests, immunodiffusion techniques, focus formation and virus neutralization techniques. These studies were in collaboration with Dr. L. R. Sibal, Dr. M. A. Fink, and Dr. K. Lee.

D. Leukemia Virus Characterization. Pools of radioactively labeled Rauscher leukemia virus of high specific activity were prepared by labeling virus produced from superinfected JLS-V9 cells established on days 5, 6, and 7 of the virus growth curve. This purified virus was used in collaborative studies with Dr. V. W. Hollis aimed at the biochemical characterization of the viral proteins (see Dr. V. W. Hollis's report, NCI-4827).

2. Study of the Humoral and Cellular Immune Response to Tumor and Oncogenic Viruses:

A. Human Studies. Four hundred and seventy-five human serums from patients with mammary adenocarcinoma, benign breast disease, leukemia, and normal controls were tested for antibodies against murine MTV. They were tested by the hemagglutination inhibition and immunodiffusion tests. Three hundred and seventy-five of these were also tested for antibodies against feline leukemia virus (Rickard), Rauscher leukemia virus, bovine serum albumin (BSA) and bovine gamma globulin (BGG). BSA and BGG antibodies were studied to determine whether they could be used as indicators of the immunological responsive status of the patient. Our data and those of others conducted in a collaborative study with a contract laboratory (69-2160) are being analysed. Our preliminary results indicate that about 10 to 20 percent of the sera tested have antibodies with HAI titers of 1:16 or higher against any one of the five antigens tested. In our hands, thus far the presence and titer of antibody to one antigen appears to be independent of that to any of the other four antigens. There appears to be no strong correlation between antibodies to any one antigen and any patient diagnosis. The antibodies in the case of FeLV and RLV appear to be specific against the virus since high titered sera (1:64 or greater) show lines of identity with standard virus and antibody in Ouchterlony plates and the human sera neutralized virus infectivity. Work must continue to clarify the significance, specificity and prevalence of these antibodies in human populations.

B. Tissue Culture Studies. The relationship of immunity to an oncogenic virus and the growth of tumor cells transformed and infected by the same virus was studied. Colony inhibition, fluorescent antibody, electron microscopy, and cytotoxicity techniques were employed. Results indicated that antibodies or immunocytes elicited by immunization with Rauscher virus are incapable of inhibiting the growth of infected and transformed cells if those cells are not actively producing viruses. The percent inhibition of colony formation was directly related to the percent of cells showing viral antigens on their surfaces by fluorescence microscopy. Inhibition of colony formation was not complement dependent.

A comparison of the sensitivity, accuracy, and ease of performance of five tests designed to measure antibody or cellular cytotoxicity was made. Superinfected JLSV-9 established cells were used as the targets for either standard BALB/c mouse, monkey or rabbit anti-RLV serum or immunocytes. The cytotoxicity of each standard serum or batch of immunocytes was titrated several times using the techniques of C_r^{51} isotope release, metabolic inhibition, colony inhibition, trypan blue uptake, and ATP release. In our hands the test of choice was Colony Inhibition. This test was the most sensitive for cellular cytotoxicity. Second choice was C_r^{51} release. Results obtained by the other three tests were too erratic to be useful.

C. Animal Studies. Studies are in progress to determine the dynamics of the development of the cellular immune response to RLV and to determine what is the most useful immunization protocol to produce a strong immunocyte response. Humoral antibodies are being measured by HAI and colony inhibition, and the cellular response is being monitored by Colony inhibition and C_r^{51} isotope release. Development of dependable immunization protocols will facilitate studies on cellular immunity and will further elucidate the relationship between humoral and cellular immunity to oncogenic viruses and tumors.

3. Immunological Models for Improved Therapy of Possible Prophylaxis of Cancer

A. Studies in Immunotherapy. Rhesus monkeys were immunized with murine leukemia virus. Thoracic duct lymphocytes were collected from 80 to 110 hours following primary immunization. A RNA-rich blastoid lymphocyte appears in large numbers (up to 20%) during this interval.

Repeated attempts were made to demonstrate some protective capability in these cells: the lymphoblasts were not directly cytotoxic to RLV producing or transformed cells by either colony inhibition or C_r^{51} release; the lymphoblasts did not protect mice challenged with tumor, leukemic cells or virus in a variety of protocols; and RNA extracted from these lymphoblasts did not protect mice from challenge with virus or cells. These results have not discouraged our aims to study transfer of immunity by RNA across species barriers. Thus, a program is underway to (i) isolate and transfer immune RNA extracted from the cells of fully immunized donors, (ii) develop a standard test system for monitoring and quantitating the transfer of immunity between animals of the same strain, and (iii) test for the transfer of immunity in the standard system by immune RNA to RLV elicited in a different species or genus. Preliminary results thus far indicate that we are able to isolate and transfer immune RNA and elicit a fairly typical immune response in a BALB/c mouse to a BALB/c mouse transfer. This experiment must be refined and standardized before we will again attempt to transfer immunity by RNA among more distant species.

B. Studies on Prophylaxis. In cooperation with Dr. George Burton, studies are underway to determine whether several oncogenic or suspected oncogenic viruses are capable of infecting and growing in a variety of insect vector cells or organs in tissue culture. Primary cultures as well as a variety of cell lines have been established. Virus infection and growth, if it occurs, will be monitored by hemagglutination titers, immunodiffusion, isotope incorporation, fluorescent and electron microscopy. This study is needed to resolve the questions of possible vector mediated epidemiology of certain cancers.

Major Findings:

1. Development of practical serologic methods for the detection and quantitation of feline leukemia virus antigens and antibodies.

2. Development of methodology for isolation of significant antigens from murine mammary tumors, leading to its application to human breast tumors.

3. There appears to be a random distribution of antibodies in the human population studied against MTV, FeLV, RLV, BSA, and BGG. Our work thus far does not show any special association of specific antibodies with a given diagnosis. Yet, certain human sera appear to contain antibodies specifically directed against one or more animal oncogenic viruses.

4. Colony inhibition is a preferable test to measure cytotoxic antibody or cellular immunity. A close second is C_r^{51} isotope release. Trypan blue, metabolic inhibition, and ATP release tests were unreliable in our hands. Lymphocyte stimulation was not tested.

Significance to Biomedical Research and the Program of the Institute.

The research approach outlined here is specifically oriented to perform experiments *in vitro*, and in animal model systems that will yield results which are directly applicable to the solution of problems of the diagnosis, etiology, treatment, or prevention of human malignancy induced by oncogenic viruses. The experiments performed fit perfectly within the program of Etiology, Viral Oncology, and the SVCP.

Proposed Course:

The goals of this project for the next fiscal year are:

1. To isolate and identify one or more tumor specific or viral antigens from human breast tumors. To produce an antiserum to at least one antigen that may permit rapid identification of good prospective specimens for study. To begin characterization of at least one isolated antigen.

2. To continue to study the question of whether animal oncogenic viruses play a discernible role in human leukemia or breast cancer.

3. To continue to search for better animal models for human tumors such as breast cancer and Burkitt's lymphoma.

4. To continue collaboration towards the characterization of the biochemical composition of the leukemia and mammary tumor viruses.

5. To develop a protocol for the reliable production of hyperimmune lymphocytes in mice to aid in the characterization of the cellular immune response to oncogenic viruses and tumors.

6. To continue exploration concerning the transfer of immunity from one species to another by the transfer of immune RNA. If successful, the immune response of the recipient must be characterized.

Honors and Awards:

None

Publications:

Sibal, L. R., Fink, M.A., Plata, E. J., Kohler, B. E., Noronha, F., and Lee, K. M.: Methods for the detection of viral antigen and antibody to a feline leukemia virus (A preliminary report). *J. Nat. Cancer Inst* 45: 607-612, 1970.

Fink, M. A., Sibal, L.R., and Plata, E.J.: Seriological detection of feline leukemia virus antigens or antibodies. In Proceedings of the Colloquium on Selected Feline Infectious Diseases held September 15-17, 1970. New York State Veterinary College, Cornell University, Ithaca. J. of Am. Veterinary Med. Assn. In press.

Plata, E. J. and Murphy, W. H.: Growth and hematologic properties of inbred BALB/Wm mice and their possible applications to studies of chronic infection. Infection and Immunity. In press.

Serial No. NCI-4830
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) Isolation and Properties of "Flat" SV40-Transformed Balb/3T3

(B) Tumorigenicity of "Flat" SV40-Transformed Balb/3T3

(C) Tumorigenicity of "Spontaneously" Transformed Balb/3T3

(D) The Induction of Cellular DNA Synthesis and the Fixation of Transformation

(E) Movement of Balb/3T3 Cells in the Absence of DNA Synthesis

(F) Viral Transformation of Hyperploid Balb/3T3

(G) Growth of Human Malignant Cells in "Factor-free" Medium

Previous Serial Number: None

Principal Investigator: Dr. Charles D. Scher

Other Investigators: Dr. Helene Smith, Bionetics Research Lab., Bethesda, Maryland
Dr. James McCoy, Bionetics Research Lab., Bethesda, Maryland
Dr. Mitchell Gail, VBB, NCI
Dr. Charles Boone, VBB, NCI
Dr. Walter Nelson-Rees, Naval Biomedical Laboratories, University of California, Oakland, California

Cooperating Units: Inside NIH
Staff, Cellular Biology Section, Viral Biology Branch, NCI

Outside NIH
Staff, Bionetics Research Laboratory, Bethesda, Maryland
Staff, Naval Biomedical Laboratories, Oakland, California

Serial No. NCI-4830

Man Years:

Total:	1.00
Professional:	1.00
Other:	0.00

Project Description

Objectives:

(A) To isolate and define the *in vitro* properties of "flat" SV40-transformed Balb/3T3 cells.

(B) To study the tumorigenicity of "flat" SV40-transformed Balb/3T3.

(C) To relate the tumorigenicity of "spontaneously" transformed Balb/3T3 to two *in vitro* growth characteristics: (1) saturation density, and (2) ability to grow in the absence of the serum growth factor(s) required by normal cells.

(D) To relate permanent SV40-transformation to the induction of cellular DNA synthesis by the virus.

(E) To learn if the movement of sparse cells is "linked" to events in the replicative cycle.

(F) To study the effects of an altered cell karyotype (hyperploid Balb/3T3) on the alteration of growth properties induced by murine sarcoma virus and SV40.

(G) To define an aspect of human malignant transformation.

Methods Employed and Major Findings:

(A) The direct isolation and properties of "flat" SV40-transformed Balb/3T3. SV40-transformants of the permanent mouse line Balb/3T3 cells are commonly selected for their ability to pile up and overgrow the "contact inhibited" monolayer of normal cells. Transformants selected by this means attain population densities 10-15 fold greater than non-transformed cells and form tumors when injected into the homologous host. (Aaronson, S.A. and Todaro, G.J.: *Science* 162: 1024-1026, 1968).

Viral-transformed Balb/3T3 that are not "contact inhibited" grow in medium lacking the serum growth factor(s) necessary to initiate DNA synthesis and cell division of nontransformed cells. In addition, "contact inhibited" viral transformants of low saturation density grow in the absence of this factor(s). Thus, growth in "factor free" medium is a criterion of viral transformation that is independent of the loss of "contact inhibition."

We have isolated SV40-transformed Balb/3T3 on the basis of their ability to grow in "factor free" medium. Twenty-nine clones were isolated and grown up into established lines. They were all transformed and had viral induced T antigen.

Their saturation densities ranged from approximately that of normal Balb/3T3 cells to tenfold higher. "Contact inhibited" SV40-transformed cells occur frequently but are not detected in the standard transformations assay.

Normal Balb/3T3 cells have a low saturation density because they cease DNA synthesis and subsequent cell division when confluence is attained. SV40 transformants continue to synthesize DNA and divide after confluence is reached and many pile up. Cellular DNA synthesis was studied by radioautography in confluent monolayers of the "contact inhibited" SV40 transformants. Two clones of "flat" transformants were found: (1) In 4/5 transformants, the majority of the cells continued to synthesize DNA in the confluent state. Many of these cells (> 10% in 72 hours) floated up into the medium. In these "flat" lines the "contact inhibited" state is a result of a large amount of cell turnover. These cells attain a saturation density at which there is an equilibrium between cell division and cell death. (2) In 1/5 "flat" transformants, the cells ceased to synthesize DNA when confluence was reached. In this line the only alteration in growth properties conferred by the virus was the ability to grow in "factor free" medium.

Primary and secondary cultures of embryonic mouse cells are diploid having forty telocentric or acrocentric chromosomes; no metacentric chromosomes are normally present. Shortly after the "crisis" period which occurred during the establishment of the permanent mouse embryo line Balb/3T3, the number of chromosomes increased to the subtetraploid level (70-80/cell) and no longer remained constant throughout the population; still few cells had metacentric chromosomes. SV40 transformants of Balb/3T3 that grow to high saturation density remain subtetraploid with few metacentrics.

"Flat revertants" of SV40-and polyoma-transformed Swiss/3T3 have been shown to be hyperploid having more than 100 chromosomes and a large increase in the number of metacentrics. "Rerevertants" that grow to high saturation density have been selected from these clonal lines; these return to the subtetraploid state and have fewer metacentrics. Thus, it is reasonable to suggest that the "contact inhibited" state of transformed cells is a result of the hyperploid state accompanied by an increased number of metacentrics. (Pollack, R., Wolman, S. and Vogel, A.: Nature 228: 938-970, 1970).

We determined the karyotype of "flat" SV40 transformed Balb/3T3. Most (3/4) of the lines that synthesized DNA at confluence remained subtetraploid. One, however, was hyperploid but with few metacentrics. The "flat" transformant that ceased to synthesize cellular DNA when confluence was reached was also hyperploid and had many metacentrics. Hyperploidy may be required to prevent SV40 transformants from synthesizing DNA at confluence; it is certainly not a prerequisite for the maintenance of the "flat" state in those lines in which DNA synthesis continued.

(B) Tumorigenicity of "flat" SV40 transformed Balb/3T3. The ability of cells to grow to a high saturation density has been correlated with their tumorigenicity. "Flat revertants" of polyoma-transformed hamster cells do not form tumors when injected into hamsters as efficiently as polyoma transformants that pile up. "Flat" clonal lines of SV40 transformed Balb/3T3 were injected into the homologous host. Preliminary evidence indicates that one line forms tumors as efficiently as an SV40 transformant that piles up. This "flat" line is hyperploid and synthesizes DNA at confluence. We are currently undertaking a more comprehensive study with these contact inhibited lines. We plan to inject them into newborn thymectomized Balb/c mice in an effort to learn if these cells have the capability to form invasive tumors and their metastases.

(C) Tumorigenicity of "spontaneously" transformed Balb/3T3 cells. We have found that all virus-transformed Balb/3T3 (SV40, MSV, RSV) lines grow in "factor free" medium, but many spontaneously transformed cells do not. The saturation densities of "spontaneous" transformants is as high as that of viral transformed lines. We are inoculating "spontaneous" transformants into Balb/c mice to learn if there is a difference in tumorigenicity between those that grow in "factor free" medium and those that do not. We plan to excise the tumors obtained and study their growth properties in vitro.

(D) The induction of cellular DNA synthesis and the fixation of transformation. When confluent cells of Swiss/3T3 were infected with a low input multiplicity of SV40 and cell division was limited by leaving the cells confluent prior to a standard transformation assay, the transformation frequency decreased tenfold. However, when cells were trypsinized and allowed one generation prior to a standard transformation assay, no transformants were lost. Thus, the fixation of the SV40 transformed state requires one cell generation after infection. (Todaro, G.J. and Green, H.: Proc. Natl. Acad. Sci. 55: 302-308, 1966). At the low input multiplicity of SV40 that these investigators used, no viral induced cellular DNA synthesis was detected.

Is viral induced DNA synthesis sufficient to "fix" transformation? Confluent Balb/3T3 cells were infected with SV40 at a multiplicity in which 60% of the cells were induced to synthesize DNA. When a standard transformation assay was performed one week later, the transformation frequency was found to have decreased more than tenfold. Thus, it is clear that DNA synthesis, per se, is not sufficient to "fix" the permanently transformed state. We are currently inactivating the virus preparation with UV-irradiation to demonstrate that the round of DNA synthesis observed was induced by SV40.

(E) Movement of Balb/3T3 cells in the absence of DNA synthesis. We have found that Balb/3T3 cells do not synthesize DNA when plated sparsely in "factor free" medium while movement continues at suboptimal levels. After the addition of serum the motion of the cells increases (commencing at 12-15 hours), followed by DNA synthesis (beginning at 18-23 hours), and mitosis (30 hours). Inhibition of DNA synthesis with cytosine arabinoside does not inhibit the movement; however, inhibition of protein synthesis by cycloheximide does. An SV40 transformed line which grows in factor free medium shows the same movement in this medium as in medium with whole serum.

(F) Viral transformation of hyperploid Balb/3T3. One clonal line of Balb/3T3 was fortuitously found to be hyperploid. Since many "flat revertants" of SV40- and polyoma-transformed cells are hyperploid, a study was undertaken to learn if these cells could be transformed. After infection of these cells with murine sarcoma virus, we found many transformants that left the monolayer. The mass cultures yielded infectious murine sarcoma virus and murine leukemia virus. We are cloning lines of these transformed cells. Infection with SV40 at an input multiplicity that transformed 15% of normal subtetraploid Balb/3T3 yielded no cells that left the monolayer. We are currently attempting to select "flat" SV40 transformants of this hyperploid line in "factor free" medium. If we obtain such transformants, it will show that SV40 became permanently associated with the cell but was incapable of causing it to pile up. This would indicate that SV40 and murine sarcoma virus act by different mechanisms to alter cellular growth control.

(G) Growth of human malignant cells in "factor free" medium. An SV40-transformed human fibroblast line grows in "factor free" medium while the parent line does not. Do malignant cells of human origin grow in this medium? Two lines of malignant cells have been tested, a fibrosarcoma and a bronchogenic carcinoma; both grow well. We are preparing to screen a larger number of normal and malignant cells. Growth in "factor free" medium may prove to be a useful means of selecting malignant cells of human origin.

Significance to Biomedical Research and the Program of the Institute:

(A) Transformants are commonly selected for their ability to pile up and leave the monolayer, or to grow in a semisolid medium (such as agar). It is important to establish other criteria of transformation. Growth in the absence of the serum growth factor(s) required by normal cells is one of these. We have selected "flat" viral transformants on the basis of their ability to grow in factor free medium, and studied their growth properties. It is important to learn why these lines have low saturation densities to provide insight into mechanisms of cellular growth control.

(B) Tumorigenicity has been correlated with the ability of cells to grow to a high saturation density. The finding that a viral transformant of low saturation density forms tumors efficiently in inbred animals suggests that other factors may be playing a role.

(C) One of the factors that may play a role in tumorigenicity is cell division in the absence of serum growth factor(s). All viral transformed cells examined to date grow in the absence of these factor(s). Many "spontaneously" transformed cells do not. To study the relationship between tumorigenicity and growth in factor free medium we used spontaneously transformed lines.

(D) An initial step in the alteration of cellular growth control by SV40 may be the induction of cellular DNA synthesis. This study is an attempt to demonstrate that viral induced cellular DNA synthesis differs from normal DNA synthesis in at least one physiologic parameter, the "fixation" of the transformation.

(E) Balb/3T3 cells synthesize DNA and move until confluence is reached; at this point both DNA synthesis and motility cease. Viral transformants continue to synthesize DNA and move in the confluent state. Is DNA synthesis "linked" to cell motility? Our data suggest that it is not since sparse Balb/3T3 cells in factor free medium do not synthesize DNA but move freely.

(F) "Flat revertants" have been isolated from polyoma- or SV40-transformed Swiss/3T3. These "flat revertants" have been shown to be hyperploid, while the parent lines that pile up are subtetraploid. We are transforming a hyperploid clonal line of Balb/3T3, with an oncogenic RNA and DNA virus, to learn if the two alter the cell's growth control by different mechanisms.

(G) Growth in the absence of the serum growth factor(s) may be a criterion of the human malignant state. In addition, it may prove to be useful in isolating cells of malignant solid tumors.

Proposed Course:

Follow the leads in the program developed during the past year and to extend them with a goal of proving a viral etiology of human cancer.

Honors and Awards:

Presented a paper entitled "Abortive Transformation of Balb/3T3 by Simian Virus 40" at the 1970 Tumor Virus Meeting at Cold Spring Harbor Laboratory, New York. The work was done in collaboration with Drs. Helene Smith and George Todaro.

Presented a paper entitled "Direct Isolation of "Flat" SV40-Transformed Balb/3T3 Cells" at the 1971 American Society of Microbiology Meeting. The work was done in collaboration with Drs. Helene Smith, Walter Nelson-Rees and George Todaro.

Publications:

Scher, C.D. and Riesz, P.: Hydrogen transfer from exchangeable to carbon bound sites in γ-irradiated dry proteins. Rad. Res. 44: 35-49, 1970.

Scher, C.D., Takemoto, K.K. and Todaro, G.J.: The resistance of SV40-transformed 3T3 to supertransformation. Proc. Soc. for Exp. Biol. & Med. In press.

Smith, H.S. and Scher, C.D.: Cell division in medium lacking serum growth factor. Comparison of lines transformed by different agents. Nature. In press.

Smith, H.S., Scher, C.D. and Todaro, G.J.: Induction of cell division in medium lacking serum growth factor by SV40. Virology. In press.

Todaro, G.J., Scher, C.D. and Smith, H.S.: SV40 transformation and cellular growth control. In Proceedings of the Ciba Foundation 1970. In press.

Serial No. NCI-4831
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on murine leukemia

Previous Serial Numbers: 4816, 4877

Principal Investigator: Dr. Ruth Merwin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To continue analysis of observations on over 200 reticulum cell neoplasms type B in BALB/c mice with regard to histological pathology, spontaneous or agent induced origin, and behavior of some in transplant.

Major Findings:

1. Reticulum cell neoplasms that had some histological characteristics of lymphocytic neoplasms were found among induced tumors. Although they were absent among primary spontaneous tumors a few arose after short latent periods among mice receiving transplants of these tumors. Lymphocytic neoplasms which are rare in old BALB/c mice also arose in mice receiving such transplants. Because the latent periods were short and there was little evidence of the presence of much effective leukemogenic agent the most likely explanation seems to be that some of the primary neoplasms were mixed and the reticulum cell neoplasm of the usual histological type predominated whereas in the new hosts, which were young, the reticulum cell tumor with some characteristics of lymphocytic neoplasms and the lymphocytic neoplasms grew better.

2. Reticulum cell neoplasms arose in mice receiving transplants of spontaneous tumors sooner than they arose in mice receiving transplants of induced ones. Although many of the tumors in mice receiving the induced tumors showed relatively long latent periods they seemed to arise from the growth of inoculated cells rather than from the induction of new tumors because they were of the same histological type as the primary tumor and some were local. Perhaps the induced tumors were more antigenic.

3. In a long transplanted tumor line originating from a spontaneous tumor most of the latent periods were either short (10 to 20 days) or long (6 to 15 months). Evidence that the tumors developing after long latent periods were not new induced ones but were from the growth of transplanted cells was: first, some of the tumors arising after transplantation were local; second, one tumor, which was transplanted to a hybrid (CAF1) produced a local tumor and it grew on transplantation back to a BALB/c; third, tumors that came up after long latent periods, including the one from a CAF1 mouse, induced transplantation resistance to each other in BALB/c mice, see 4. This would not seem likely to occur if the tumors were newly induced. Carswell, Wanebo, and Old found that each spontaneous reticulum cell tumor in SJL/J strain mice induced resistance that was specific against itself.

4. A spontaneous tumor transplanted for many generations yielded a number of tumors that arose after long latent periods. Sublines developed from these tumors induced resistance against the transplantation of the other sublines. Irradiated cells were used for immunization. These tumors did not immunize against nor respond to immunization by another reticulum cell tumor that arose in a mouse carrying a leukemogenic agent. It appeared that some sublines lost some of their antigenicity and others lost some of their capacity to respond to immunity. Thus, for instance, one subline immunized well against itself and against another subline but poorly against a 3rd line. But the 3rd line immunized against the first line although poorly against itself.

Significance to Biomedical Research and the Program of the Institute:

The etiology of leukemias in BALB/c mice where the tumors arise spontaneously in the absence of a high concentration of a leukemogenic agent may be similar to the etiology of leukemias in humans. Also knowledge of conditions leading to the growth of reticulum cell tumors in mice many months after they receive transplants may be of significance in understanding some of the recurrences of leukemias after remissions caused by chemotherapy.

Proposed Course:

1. To ascertain whether lymphocytic neoplasms are, as suggested here, present but inhibited in their growth in old mice with spontaneous reticulum cell neoplasms. If this proved to be the case the reasons for the inhibited growth would be investigated.

2. To study the conditions leading to the growth of tumors many months after transplantation.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4834
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) *In Vitro* and *In Vivo* Studies of Herpes Virus Saimiri

(B) Human Rauscher Virus

(C) Cytomegalovirus (CMV) of Human and Subhuman Species

Previous Serial Numbers: 4873, 4890

Principal Investigator: Dr. Dharam V. Ablashi

Other Investigators:
Mr. G.R. Armstrong
Dr. W. Turner
Dr. U. Heine
Dr. H.C. Chopra
Dr. A.J. Dalton
Miss E. Blackham
Dr. W.F. Loeb
Dr. M.G. Valerio
Mr. D. Bennett

Cooperating Units: Inside NIH
Microbiology Section, VBB, NCI
Ultrastructure Studies Unit, ET, NCI
Electron Microscopy Section, VBB, NCI

Outside NIH
Bionetics Research Laboratories, Kensington, Maryland

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

(A) *In vitro* infectivity of Herpes virus saimiri (HVS) for human and subhuman cell cultures. *In vivo* infectivity of HVS propagated in human and subhuman cell cultures.

(B) In vitro and in vivo characterization of Rauscher leukemia virus propagated in human cell cultures (HRV).

(C) To isolate and identify virus from (1) human tumors of lymphoid origin established cell culture; (2) from oral and anal orifices of owl monkeys selected to be used for Herpes virus saimiri work.

Methods Employed:

(A) Primary continuous cells of human, old and new world monkeys were employed. The viral preparations were studied for biological and immunological properties. The animals were checked for HVS virus, antibody, hematological and pathological findings. Electron microscopy was used for virus identification.

(B) A continuous cell culture of human origin (HEK) with other human and mouse cell cultures were used. Immunological tests (neutralization and complement fixation) were performed. NIH Swiss and Balb/c mice were used.

(C) Inoculation of human and monkey tissue cultures. Electron microscopy examination of infected cells. Biological characterization of the virus isolated from these cells.

Major Findings:

(A) HVS showed replication in human subhuman cell cultures. The yields of HVS from African monkey (AG) cells were parallel to yields from owl monkey (OM) cells; however, cells of human and Rhesus monkey origin were quite lower than OM and AG cells.

The biological and immunological properties showed that HVS from human and old world monkey cells were indistinguishable from HVS from OMK cells.

The HVS particles in different stages of maturation were found in human and subhuman infected cells; however, infected tumors of inoculated animals failed to show any virus. Same tissues upon co-cultivation revealed HVS.

Two monkeys inoculated with AGMK-HVS developed malignant lymphoma with lymphocytic leukemia. The animals lived longer and showed more differentiated blood malignancy. The tissue also showed no necrosis or giant cells.

(B) Two lots of Hazelton RLV (HRLV) replicated in primary and continuous human cell cultures. The early passages of HRLV formed to infect human and mouse embryo cells, but later passages failed to infect mouse cells.

The in vitro and in vivo immunological data indicated that anti-HRV failed to neutralize RLV but reacted with ether treated RLV indicating a common GS antigen.

The XC test demonstrated that 11th passage of HEK-HRV is negative whereas 10th passage HRV was positive by same test.

The 5th passage of HEK-HRV induced leukemia in mice and same passage transferred to 3T3 and NIH Swiss mouse cell also induced leukemia in mice. The 20th passage of HEK-HRV was negative for in vitro and in vivo mouse infectivity.

The HRV rescued MSV genome from HT cells which induced morphological alterations in WI-38 and HEK cells. The foci of altered cells were inhibited in presence of anti-HRV serum.

H-RLV once passaged through Balb/c mice was still infective to human cell cultures.

The animals inoculated with late passage HEK-HRV infected cells failed to show any protection against RLV.

(C) Human CMV. The lymphoid cell was co-cultivated with WI-38 cells and a Herpes virus was rescued. These cells by EM showed no virion except inclusions for a period of eight months. The isolate according to Herpes and classification is a CMV. Its further immunological studies showed that it is closely related to AD-169 strain of human CMV.

Owl Monkey CMV. The oral and anal swabs of an adult owl monkey revealed the presence of a Herpes virus. The virus is a member of Herpes B group virus and further data classified it as a CMV. The serological studies so far showed that it is widely distributed in owl monkey population (70% contain antibodies). The experimental sera from human (AD-169) African green (SA6) and Rhesus CMV isolate did not neutralize this isolate. The sera also failed to show any CF titers to this CMV isolate. No antibodies to this isolate were detected in Rhesus and African green population.

Significance to Biomedical Research and the Program of the Institute:

(A) Virus can replicate human and old monkey cells. The EM findings suggest virus may be defective in stage in tissues. The study has significance in chemotherapy and other related studies with human cancer such as Burkitt lymphoma.

(B) The data indicate either another "C" type infecting human cells or attenuation of RLV. The rescue ability of HRV should be used to rescue "C" type viruses from human tumors. The XC data can be used in comparing HRV for biochemical and biological analysis.

(C) The study first establishes that to our knowledge this is the first CMV isolate from an owl monkey. The isolation from oral and anal orifices further show that this route should not be overlooked for virus isolations. Previously, CMV's have been isolated from tissues and urine.

Proposed Course:

(A) The studies will be continued.

(B) Further *in vivo* and *in vitro* studies will be continued.

(C) Co-cultivation of long established tumor cell lines will be tried to rescue virus. The owl monkey CMV will be further tested for *in vitro* and *in vivo* pathogenicity.

Honors and Awards:

Presented paper at American Society for Microbiology, 1970, Title: Murine Cytomegalovirus: Inhibition of Splenomegaly Induced by Rauscher Plasma Virus in Mice. J.M. Easton, D.V. Ablashi, A. Simmons and D.E. Waggoner. Abstract #V34 ASM Proceedings.

Author on paper presented at American Society for Microbiology, 1970, Title: Production of Interferon *in vitro* by Cell Infected with Murine Cytomegalovirus. H.K. Oie, J.M. Easton, D.V. Ablashi and S. Baron. Abstract #V47.

The following papers have been selected for presentation at the 1971 meetings of the American Society for Microbiology, FASEB, American Cancer Society:

1. *In Vitro* and *In Vivo* Characterization of Rauscher Leukemia Virus Propagated in Human Cells (HRV). D.V. Ablashi, G.R. Armstrong, S.L. Myers and E.A. Blackham. ASM Paper #V295.

2. Rescue of Defective Murine Sarcoma Virus (MSV) Genome with Rauscher Leukemia Virus Propagated in Human Cell Cultures (HRV). D.V. Ablashi and G.R. Armstrong. ASM Paper #V292.

3. Physiochemical, Immunological and Infectivity Studies of Herpes virus saimiri (HVS) Propagated in Human and Primate Cells. D.V. Ablashi, E. Blackham and V. Heine (FASEB). (This paper is also selected by FASEB Society for press release).

4. Morphologic Studies on Herpes virus saimiri in Subhuman and Human Cell Cultures. U. Heine, D.V. Ablashi and G.R. Armstrong. (American Cancer Society).

Publications:

Heine, U., Kondratick, J., Ablashi, D.V., Armstrong, G.R. and Dalton, A.J.: Ultrastructure and biologic properties of a cytomegalo virus rescued from human paraganglioma. Cancer Research. In press.

Levine, P.H., Ablashi, D.V., Bernard, C.W., Carbone, P.P., Waggoner, D.E. and Malan, L.: Elevated antibody titers to Epstein-Barr virus in Hodgkin's disease. Cancer 27: 416-421, 1971.

Martos, L.M., Ablashi, D.V., Gilden, R.V., Siguenza, R.F. and Hampar, B.: Preparation of immune rabbit sera with neutralizing activity against human cytomegalovirus and varicellazoster virus. J. Gen. Virol. 7: 169-171, 1970.

Serial No. NCI-4835
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A. Studies of cell lines infected with murine sarcoma virus in absence of MLV: rescue phenomenon and evidence for production of a defective virus-like particle.

B. Investigation of the biological infectivity and polymerase activity of a hamster-derived MSV

C. Virus search in a Wilm's Tumor cell line

D. Epidemic hepatitis among institutionalized mentally retarded children

E. Epidemiologic investigation of a reported increase in lymphosarcoma cases from Eastern Virginia

Previous Serial Number: None

Principal Investigator: Dr. Paul T. Peebles

Other Investigators:
Dr. Adi Gazdar, VLLB, NCI
Dr. Robert H. Bassin, VLLB, NCI
Dr. Leo Phillips, VLLB, NCI
Dr. Daniel Haapala, VLLB, NCI
Dr. Willard Helmuth, NCDC, Atlanta
Dr. Samuel Baron, NIAID

Cooperating Units: Inside NIH:
Staff of the VLLB, NCI

Outside NIH:
Staff of NCDC, Atlanta, Viral Disease Section and Epidemiology

Man Years:

Total: 3.0
Professional: 1.0
Other: 2.0

Serial No. NCI-4835

Project Description

Objectives:

A. To study rescue phenomena from cell lines infected with murine sarcoma virus (MSV) in the absence of murine leukemia virus (MuLV) and to characterize defective virus-like particles produced from these lines.

B. To investigate the biological infectivity and RNA dependent-DNA polymerase activity of a mammalian MSV.

C. To search for a virus in a Wilm's Tumor cell line.

D. Investigate two epidemics of hepatitis in institutions for the mentally retarded in Virginia.

E. To investigate a reported increase of lymphosarcoma cases from Eastern Virginia.

Methods Employed:

A.-C. Sarcoma and leukemia virus production in cell culture were measured by known tissue culture assays, tritiated uridine labelling, immunological tests, and electron microscopy. The requirement for DNA synthesis in virus propagation was determined by assaying virus production with and without DNA synthesis inhibitors.

D. Epidemological investigation of two hepatitis outbreaks that occurred in institutions for the mentally retarded in Lynchburg and Petersburg, Virginia was augmented by the use of clinical surveillance and the evaluation of serial sera from a random sample of the population for the presence of Australian antigen and elevation of SGOT. Mass intramuscular injection of gamma globulin was used for prophylaxis.

E. Epidemiological investigation of the possible increase of lymphosarcoma cases in Eastern Virginia was performed by study of the cases, recording demographic and epidemiologic data, and subsequent comparison of the observed incidence with the expected which was computed from the vital statistics from the 1960 census data for the counties involved.

Major Findings:

A. The isolation of cloned cell lines infected with MSV in the absence of detectable MuLV has afforded the opportunity to study the defectiveness of MSV because in these cells MSV is unable to replicate. Furthermore, the development of a quantitative MuLV assay permits the study of MuLV kinetics.

Superinfection of a cloned (S+L- cell) line with MuLV resulted in a logarithmic rise in titer of both MSV and MuLV in the supernatant, beginning at 12 hours and becoming maximum at 72 hours after superinfection. MSV was not produced without the simultaneous release of progeny MuLV. Also MSV was released in excess of the initial log phase of production. This MSV excess permitted the isolation of MSV from MuLV by simply titrating out past the end-point of MuLV to where there was still detectable MSV. Applying these dilutions to 3T3 mouse monolayers pretreated with DEAE dextran resulted in the formation of S+L- cell lines. It remains to be determined whether these cells are non-producers or producers. They release no competent virus until they are superinfected with MuLV, after which they release both MuLV and MSV.

The inhibition of DNA synthesis during the period of eclipse between superinfection and the emergence of compent MSV resulted in a decrease in the production of both virus types.

In order to further characterize these S+L- cells, the original cloned lines were studied and found to be releasing a C-type virus-like particle which was seen on EM and also at a 1.16 buoyant density in a sucrose gradient. This particle lacks biological infectivity on conventional cell lines, even after co-sedimentation with MuLV.

B. The MSV hamster derived tumor cell lines HTG, and HTG2 produce large quantities of non-infectious type C particles with a buoyant density of 1.16 and are antigenically related to the original murine virus. Competent focus forming MSV could be isolated from these lines when they were co-cultivated with mouse embryo fibroblasts and superinfected with MuLV. Purified virus preparations from HTG2 have either minimal or no detectable RNA dependent DNA polymerase activity and this finding may explain lack of infectivity of this virus. Further studies on the physical-chemical properties of this virus-like particle as well as its biological activity are in progress.

C. An established Wilm's Tumor line with intracellular papova virus-like particles has in previous studies been negative for biological and immunological activity. Consequently, similar studies are in progress on virus preparations from cell associated material.

D. At the Lynchburg Training School in Lynchberg, Virginia, there was an epidemic of short incubation infectious hepatitis between April and September 1970. Among the 3,600 population, there were 370 clinical cases and far more subclinical cases. The severely retarded ambulatory children had the highest attack rate because of the greater chance of fecal-oral spread. Epidemiologic investigation allowed confinement of the patient workers and the children to their wards, and mass gamma globulin prophylaxis contributed to ending the epidemic.

The epidemic was not related to Australian antigen which was found to be unchanged with an incidence of 7% in the population.

Several cases of clinical hepatitis began to appear in the children's unit of the Central State Hospital in Petersburg, Virginia, and 8 of the 12 clinical cases were confined to one ward whereas the other 4 cases were from three other wards. Blood studies revealed that 88% of that particular ward had hepatitis.

Epidemiological investigations revealed that a patient from the Lynchburg epidemic was unintentionally transferred to Central State Hospital while manifesting clinical hepatitis.

The epidemic resulted in a total of 42 cases of hepatitis, 13 of which were clinical. Previous experience from Lynchburg, which had determined that patient workers and social contacts were vectors of spread, prompted the rapid confinement of the workers and children to their wards. Gamma globulin prophylaxis was given to all the children. A random sample from the population is being followed for clinical illness along with blood samples for SGOT values and Australian antigen at frequent intervals. Initial results would suggest that there was an epidemic of short incubation infection type hepatitis superimposed on a population with endemic Australian antigen at a rate of 24%, which is high for a non-Mongoloid population. The incidence of Australian antibody in the sample was 7%, also quite high. There were patients who were Australian antigen positive who were not immune to acquiring the short incubation hepatitis.

Preliminary epidemiological evidence would suggest that the severely retarded fully ambulatory patients had a greater chance for being Australian antigen positive as compared to severely retarded bedridden. This would support the hypothesis that Australian antigen type hepatitis may be spread by the fecal-oral route. Furthermore, although it is uncommon to be able to stop a hepatitis epidemic in this type institution, rapid epidemiology determining source and spread as well as mass gamma globulin prophylaxis prevented the epidemic from continuing.

E. In October of 1970, it was reported that 11 cases of lymphosarcoma had been diagnosed in the past year in the eastern portion of Virginia. Epidemiologic investigation was undertaken and revealed that in the past year, there had been a total of 19 cases of leukemia and lymphoma from the eastern counties of Virginia. The observed number of lymphoma cases were 2 to 5 times that expected. However, if the observed cases of leukemia and lymphosarcoma were combined and compared to the expected number of cases from the population, then the observed number was less rather than greater than the expected. There was no particular clustering of cases except for 2 cases of lymphosarcoma from the town of Colonial Heights. These two cases representing a 15 to 70 times greater incidence than expected for the population, will be further investigated for any epidemiological relationship. The overall increase in incidence of lymphomasarcoma in Eastern Virginia remains unexplained.

Significance to Biomedical Research and the Program of the Institute:

The exploration of oncogenic RNA viruses, their defectiveness, and the variance between their capacity to replicate and their capacity to transform has great implications in that their existence makes probable the viral etiology for human cancer, and that when the human oncogenic viruses are isolated they are likely to function in a manner analogous to the mammalian oncogenic viruses, furthermore, facilitating the isolation of the human viruses. The ability to quickly produce S+L- cells may serve as a method for producing a human cell line with a sarcoma genome that would release detectable virus when superinfected with a human leukemia virus, and a cell line of this type could be used for detecting human leukemia viruses.

Epidemiological investigations of human lymphosarcoma clusters may lead to the discovery of factors that are important in the etiology of the spread of the disease.

Proposed Course:

A. Currently we are involved in cloning and characterizing the S+L- cells obtained by the end-point titration method. This rather quick method for isolating S+L- cells may also be an asset for the development of an MuLV assay in the BALB/3T3 mouse cell lines to facilitate the rapid study of MuLV tropism. Rescue of the MSV, which is not detectable without MuLV superinfection, may prove to be an ideal approach to the study of the effect of inhibitors on virus replication in vitro, and further studies of the requirement of cellular DNA synethesis in rescue phenomena are planned.

B. The isolation of a variety of non-competent virus-like particles released from cells which have only the MSV genome present in them possibly affords the opportunity for the study of MSV defectiveness by determining which functions must be present for competent transformation and which are required for replication.

C. It is our intention to continue to search for viruses from human tumors and to demonstrate the oncogenic capacity of the virus.

Serial No. NCI-4835

Honors and Awards:

None

Publications:

None

Serial No. NCI-4836
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanisms of Virus-Induced Transformation

Previous Serial Number: NCI-4914

Principal Investigator: Dr. Stuart A. Aaronson

Other Investigators: Dr. Jeffrey Ross
Dr. John Stephenson
Dr. Edward Scolnick
Dr. George J. Todaro

Cooperating Units: Inside NIH
Dr. Wade Parks, VCB, NCI
Dr. Wallace P. Rowe, LVD, NIAID
Dr. Robert Huebner, VCB, NCI
Dr. Malcolm Martin, LBV, NIAID
Dr. Robert Bassin, VLLB, NCI

Outside NIH
Dr. David Lytle, Environmental Health Bureau, Rockville, Maryland
Dr. Harvey Dosik, Maimonides Hospital, Brooklyn, New York
Dr. John Kersey, University of Minnesota, Minneapolis, Minnesota
Dr. Raymond Gilden, Flow Laboratories, Rockville, Maryland
Dr. Leonard Hayflick, Stanford University, Stanford, California
Dr. Roger Wilsnak, Huntington Research Laboratories, Baltimore, Maryland
Naval Biological Laboratory, Oakland, California

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Serial No. NCI-4836

Project Description

Objectives:

1. To study the mechanism of transformation by RNA and DNA containing tumor viruses.

2. To develop quantitative systems to study the factors effecting transformation in mouse cell lines and in human cell strains.

3. To apply the methods to the search for possible transforming viruses of man.

Methods Employed:

Standard cell culture procedures of this laboratory. Enzymology, nucleic acid homology studies, and other biochemical techniques.

Major Findings:

1. Continuous lines of BALB/c and NIH Swiss mouse cell lines have been developed with which to study normal growth control mechanisms and the effects of RNA and DNA tumor viruses on these mechanisms.

2. MSV-transformed BALB/3T3, NIH/3T3 and rat cell lines have been demonstrated to contain the sarcoma genome in a non-replicating state. These nonproducer (NP) lines are extremely tumorigenic and provide an excellent experimental model for human malignancy. Investigations are being carried out as to the state of the viral genetic information in these cells and whether the cells contain a viral RNA-dependent DNA polymerase. The surface antigens of the NP cells are also being investigated.

3. Human cells can be transformed by the RNA tumor virus, murine sarcoma virus, and by the DNA tumor virus, SV40. A study of the mechanism of enhanced viral susceptibility of cells from patients with certain disease state is in progress. With SV40, it has been found that the use of purified viral DNA eliminates differences in transformation seen with whole virus, suggesting that the block to transformation in resistant cells resides in a step prior to complete virus uncoating.

4. Studies of radiation-virus cocarcinogenesis have revealed that UV damaged SV40 can be repaired by the host cell, thus demonstrating for the first time, host-cell reactivation of a tumor virus by mammalian cells.

5. A genetic analysis of the murine C-type RNA tumor viruses is being approached by selection techniques to obtain virus mutants.

6. Transformation of human and mouse cells by isolated SV40 DNA has been accomplished. The potential application of integrating genetic information by means of introducing DNA into cells is being investigated.

7. New techniques have been developed in virology including rapid assays for SV40 infection and mycoplasma detection. The ability to grow human tumor cells from primary explants of tumor tissue by a selection technique based on lack of contact inhibition has been demonstrated.

8. A study of the biochemistry of RNA-tumor viruses has revealed that two additional groups of viruses contain an RNA-dependent DNA polymerase, visna and foamy viruses. Whether these latter viruses are etiologically involved in cancer in their natural hosts is currently being studied. RNA-dependent DNA polymerase activity has also been detected in normal human and mouse cells as well as in tumor cells. The relationship between these enzymes and the enzyme of known RNA tumor viruses is being investigated. An antibody to the viral enzyme has been demonstrated in sera of rats with MSV-induced tumors and has also been produced by immunization of rabbits with partially purified murine viral polymerase. These antibodies also neutralize other mammalian C-type viruses but not MTV or the avian tumor viruses.

9. The techniques described above are being rapidly applied to the search for a viral etiology of human cancer.

Significance to Biomedical Research and the Program of the Institute:

The systems that are being intensively investigated have provided a much better understanding of the biology and biochemistry of viral transformation. It is felt that a clear understanding of these phenomena will significantly speed the progress in our search for a viral etiology of human cancer.

Proposed Course:

Follow the leads in the program developed during the past year and to extend them with a goal of proving a viral etiology of human cancer.

Honors and Awards:

None.

Publications:

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Aaronson, S.A.: Susceptibility of human cell strains to transformation by SV40 and SV40 DNA. J. Virology 6: 470-475, 1970.

Aaronson, S.A.: Human cell transformation by SV40 and SV40 DNA. McGraw-Hill Yearbook of Science and Technology. In press.

Aaronson, S.A.: Isolation of a rat-tropic leukemia virus from M-MSV-O. Virology. In press.

Aaronson, S.A. and Lytle, C.: Decreased host cell reactivation of irradiated SV40 virus in xeroderma pigmentosum. Nature 228: 359-361, 1970.

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Aaronson, S.A. and Rowe, W.P.: Nonproducer clones of murine sarcoma virus transformed BALB/3T3 cells. Virology 42: 1-8, 1970.

Aaronson, S.A. and Todaro, G.J.: Infectious SV40 and SV40 DNA: Rapid fluorescent focus assay. Proc. Soc. Exp. Biol. & Med. 134: 103-106, 1970.

Aaronson, S.A., Jainchill, J.L. and Todaro, G.J.: Murine sarcoma virus transformation of BALB/3T3 cells: Lack of dependence on murine leukemia virus. Proc. Natl. Acad. Sci. U.S. 66: 1236-1243, 1970.

Aaronson, S.A., Parks, W.P., Scolnick, E.M. and Todaro, G.J.: Antibody to the RNA-dependent DNA polymerase of mammalian C-type RNA tumor viruses. Proc. Natl. Acad. Sci. U.S. In press.

Aaronson, S.A., Todaro, G.J. and Freeman, A.E.: Human sarcoma cells in culture: Identification by colony-forming ability on monolayers of normal cells. Exp. Cell Research 61: 1-5, 1970.

Aaronson, S.A., Todaro, G.J. and Huebner, R.J.: Transformation by murine sarcoma virus. 2nd Lepetit Colloquium Proceedings. In press.

Gerwin, B.I., Todaro, G.J., Zeve, V., Scolnick, E.M. and Aaronson, S.A.: Separation of RNA-dependent DNA polymerase activity from the murine leukemia virion. Nature 228: 435-438, 1970.

Kelloff, G., Aaronson, S.A. and Gilden, R.V.: Inactivation of murine sarcoma and leukemia viruses by ultraviolet irradiation. Virology 42: 1133-1135, 1970.

Parks, W.P., Scolnick, E.M., Todaro, G.J. and Aaronson, S.A.: RNA-dependent DNA polymerase in primate syncytium-forming ("foamy") viruses. Nature 229: 258-260, 1971.

Scolnick, E.M., Aaronson, S.A. and Todaro, G.J.: DNA synthesis by RNA tumor viruses. Proc. Natl. Acad. Sci. U.S. 67: 1034-1041, 1970.

Scolnick, E.M., Aaronson, S.A., Todaro, G.J. and Parks, W.P.: RNA dependent DNA polymerase activity in mammalian cells. Nature 229: 318-321, 1971.

Scolnick, E.M., Rands, E., Aaronson, S.A. and Todaro, G.J.: RNA-dependent DNA polymerase activity in five RNA viruses: Divalent cation requirements. Proc. Natl. Acad. Sci. U.S. 67: 1789-1796, 1970.

Stone, L.B., Scolnick, E.M., Takemoto, K.K. and Aaronson, S.A.: Visna virus: A slow virus with an RNA dependent DNA polymerase. Nature 229: 257-258, 1971.

Todaro, G.J., Aaronson, S.A. and Rands, E.: Rapid detection mycoplasma-infected cell cultures. Exp. Cell Research. In press.

Todaro, G.J., Aaronson, S.A., Scolnick, E.M. and Parks, W.P.: RNA-dependent DNA polymerase in viruses and in cells. 2nd Lepetit Colloquium Proceedings. In press.

Todaro, G.J., Zeve, V. and Aaronson, S.A.: Cell culture techniques in the search for cancer viruses of man. In Vitro. In press.

Worthington, M. and Aaronson, S.A.: The interferon system in cells from human tumors and from persons predisposed to cancer. Infection and Immunity. In press.

Serial No. NCI-4837
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemistry of RNA Tumor Viruses

Previous Serial Number: None

Principal Investigator: Dr. Edward M. Scolnick

Other Investigators: Dr. Stuart A. Aaronson
Dr. Jeffrey Ross
Dr. George J. Todaro

Cooperating Units: Inside NIH
Dr. Wade Parks, VCB, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. The virions of RNA tumor viruses, visna virus and foamy viruses have been found to contain enzymes capable of synthesizing DNA from an RNA template. Our efforts have been in two general directions:

a. to understand the enzymology of the reactions; and
b. to be able to use the enzymes as potential probes into the cause of human cancer.

Methods Employed:

Standard biochemical purification and analytical techniques.

Major Findings:

1. Mechanism of reaction. Virions contain at least five enzymatic activities. An RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase, a DNA endonuclease, a DNA exonuclease and a DNA ligase. We have found that with the Rauscher strain of murine leukemia virus manganese

is very important as the divalent cation. Using manganese and various RNA·RNA and RNA·DNA synthetic polymers it has been possible to greatly increase the sensitivity of detection of the polymerase(s). The usefulness of this increase sensitivity will be outlined below.

Assays have also been set up for the endonuclease and exonuclease activities, and attempts are now being made to define the number of enzymes involved in DNA synthesis and how they interact. This information will make it easier to interpret the meaning of any RNA-dependent DNA polymerase activity found in human cancer cells.

2. Detection of enzymes in cells. The enzymes found in RNA tumor viruses provide potential markers for the presence of such viruses in cells. The advantages of such biochemical markers is the rapidity with which assays can be run, their sensitivity, and their ability at least potentially to detect virions which might be difficult to grow. Using manganese and synthetic RNA-DNA templates, RNA-dependent DNA polymerase has been found in mouse cells transformed by Moloney sarcoma virus, and African Green monkey kidney cells infected with a foamy virus. These are then useful model systems for the study of various human disease states. During the course of searching for enzyme in human tumors, RNA-dependent DNA polymerase activity has been found in human leukemic cells. However, surprisingly, activity has also been found in normal mouse and human fibroblasts. Thus the detection of activity per se does not allow us to attribute this enzyme to a presumptive virus in these cells. Attempts are now in progress to develop ways to distinguish the known RNA-dependent DNA polymerase from mammalian C-type virions from the activity found in normal and tumor cells. Methods to purify, separate and compare the enzymes are being developed. Antibodies are being made against the viral and different cellular enzymes in an attempt to see if they are immunologically related.

Significance to Biomedical Research and the Program of the Institute:

It is hoped that the above approaches will allow us to distinguish viral from cellular enzymes and therefore provide a very sensitive biochemical marker in the search for viral etiology to human cancer.

Proposed Course:

Follow the leads in the program developed during the past year and to extend them with a goal of proving a viral etiology of human cancer.

Honors and Awards:

None.

Publications:

Scolnick, E.M.: Preparation of *E. coli* polypeptide termination factors. Manual in Molecular Biology. In press.

Scolnick, E.M., Aaronson, S.A. and Todaro, G.J.: DNA synthesis by RNA tumor viruses. Proc. Nat. Acad. Sci. U.S. 67: 1034-1041, 1970.

Scolnick, E.M., Aaronson, S.A., Todaro, G.J. and Parks, W.P.: RNA dependent DNA polymerase activity in mammalian cells. Nature 229: 318-321, 1971.

Aaronson, S.A., Parks, W.P., Scolnick, E.M. and Todaro, G.J.: Antibody to the RNA-dependent DNA polymerase of mammalian C-type RNA tumor viruses. Proc. Nat. Acad. Sci. U.S. In press.

Caskey, C.T., Scolnick, E.M., Tompkins, R., Milman, G. and Goldstein, J.: Release factors: *In vitro* assay and purification methods in enzymology. In press.

Gerwin, B.I., Todaro, G.J., Zeve, V., Scolnick, E.M. and Aaronson, S.A.: Separation of RNA-dependent DNA polymerase activity from the murine leukemia virion. Nature 228: 435-438, 1970.

Parks, W.P., Scolnick, E.M., Todaro, G.J. and Aaronson, S.A.: RNA-dependent DNA polymerase in primate syncytium-forming ("foamy") viruses. Nature 229: 258-260, 1971.

Scolnick, E.M., Rands, E., Aaronson, S.A. and Todaro, G.J.: The divalent cation requirements for RNA-dependent DNA polymerase activity in five RNA viruses. Proc. Nat. Acad. Sci. U.S. 67: 1789-1796, 1970.

Stone, L.B., Scolnick, E.M., Takemoto, K.K. and Aaronson, S.A.: Visna virus: A slow virus with an RNA-dependent DNA polymerase. Nature 229: 257-258, 1971.

Todaro, G.J., Aaronson, S.A., Scolnick, E.M. and Parks, W.P.: RNA-dependent DNA polymerase in viruses and in cells. 2nd Lepetit Colloquium Proceedings. In press.

Serial No. NCI-4838
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on the Genetic Relationships Between Murine Leukemia and Sarcoma Viruses

Previous Serial Number: None

Principal Investigators: Dr. John R. Stephenson

Other Investigators:
Dr. Stuart A. Aaronson
Dr. Jeffrey Ross
Dr. Edward Scolnick
Dr. George J. Todaro

Cooperating Units: Outside NIH
Dr. A.A. Axelrad, University of Toronto
Dr. D.L. McLeod, University of Toronto

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To develop micro techniques for the rapid screening of mutagenized leukemia virus stocks in order to obtain temperature sensitive mutants to be used to study leukemia virus replication.

2. To determine the genetic relatedness between murine leukemia and sarcoma viruses, and to examine the state of these viruses in both murine and human transformed cells.

3. To study the phenotype expression of the sarcoma virus genome in MSV-transformed "non-producer" mouse cells.

Serial No. NCI-4838

Methods Employed:

Standard tissue culture procedures, including XC-plaque assays, for leukemia virus and focus formation assays for sarcoma virus, nucleic acid hybridization and *in vivo* tumor rejection.

Major Findings:

1. Micro techniques for isolation of temperature sensitive leukemia virus mutants have been developed.

2. The genetic relationship between two Kirsten leukemia and Kirsten sarcoma virus has been examined. *In vitro* DNA products were synthesized from the respective viral RNA templates by use of the recently discovered RNA-dependent DNA polymerase. By reciprocal RNA-DNA hybridization experiments, large differences in the genetic information of the two viruses was demonstrated.

3. Development of *in vitro* hemopoietic culture systems for the study of murine leukemia viruses.

Significance to Biomedical Research and the Program of the Institute:

Isolation of leukemia virus temperature sensitive mutants should lead to a better understanding of replication and transformation by viruses of the leukemia-sarcoma complex. The lack of homology between Kirsten leukemia and sarcoma virus is significant for performing and interpretating experiments in which viral *in vitro* DNA products are used as a probe for the detection of viral information both in mouse and in human transformed cells.

Proposed Course:

Follow the leads in the program developed during the past year and to extend them with a goal of proving a viral etiology of human cancer.

Honors and Awards:

None.

Publications:

Stephenson, J.R. and Axelrad, A.A.: Separation of erythropoietin sensitive cells from hemopoietic spleen colony-forming cells of mouse fetal liver by unit gravity sedimentation. *Blood*. In press.

Stephenson, J.R. and Axelrad, A.A.: Quantitative assay method for erythropoietin *in vitro*. *Endocrinology*. In press.

Stephenson, J.R., Axelrad, A.A., McLeod, D.L. and Shreeve, M.M.: Induction of colonies of hemoglobin-synthesizing cells by erythropoietin *in vitro*. *Proc. Natl. Acad. Sci.* In press.

Serial No. NCI-4839
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A. Use of a continuous cat cell line (Crandell) for studies with feline leukemia viruses and their corresponding murine sarcoma virus pseudotypes

B. The adaptation of microplate cell culture techniques for the assay and cloning of murine leukemia and sarcoma viruses

C. Virus replication and chronic infection of 3T3 cells following exposure to radiation-induced mouse leukemia virus (Rad LV)

D. Determination of relative portions of the murine leukemia virus (MuLV) genome required for virus replication and for rescue of defective murine sarcoma virus (MSV)

Previous Serial Number: None

Principal Investigator: Dr. Shigeko Nomura

Other Investigators: Dr. Peter J. Fischinger, VLLB, NCI
Dr. Robert H. Bassin, VLLB, NCI
Dr. Kyu M. Lee, VLLB, NCI
Dr. Willie Turner, VLLB, NCI

Cooperating Units: Inside NIH
Staff of Viral Leukemia and Lymphoma Branch, NCI

Outside NIH
None

Man Years:

Total: 1.0
Professional: 1.0
Other: 0.0

Project Description

Objectives:

A. To develop quantitative assay techniques for feline leukemia viruses and the corresponding MSV pseudotype, MSV(FelLV), using a continuous feline cell line (Crandell).

B. To adapt microplate techniques for the cloning and assay of murine leukemia viruses based on the induction of lytic foci in S+L- cells and to analyze the products of single virus cell interactions.

C. To study quantitative aspects of the replication of Rad LV using chronically infected 3T3 and S+L- cell lines.

D. To compare the relative size of the MuLV genome required for virus replication with that necessary for rescue of "defective" MSV from transformed 3T3 cells.

Methods Employed:

Routine tissue culture methods and standard virological techniques for the quantitation of cell transformation and focus formation by mammalian sarcoma and leukemia viruses were used. Relative genome sizes were determined following stepwise inactivation with ultraviolet light (UV).

Major Findings:

A. A careful comparison of the Crandell cat cell (CCC) line and feline embryo cells (FEF) with regard to their sensitivities to infection and transformation by feline leukemia virus (FelLV) and MSV(FelLV) was undertaken. The CCC line was found to be slightly less sensitive than FEF to focus formation by MSV(FelLV) and to the "helper" activity of FelLV itself. DEAE-dextran exerted an enhancing effect on titers of both viruses in these cells, comparable to other RNA tumor-virus cell systems. Interference assays based on the resistance to superinfection with MSV(FelLV) in the CCC previously infected with FelLV could be carried out, giving a good measure of the number of replicating units of FelLV in a given virus stock. The CCC line is ideal for interference assays, since several cell passages are often required for maximum sensitivity.

B. Focus induction in clonally purified S+L- mouse cells by MuLV could be demonstrated using microplates. End point dilution estimates of MuLV focus-inducing activity in microplates were approximately 8-fold lower than titers determined by enumeration of lytic foci in petri dishes. The usefulness of this system as a cloning procedure for MuLV is being evaluated. Transformation of 3T3 cells by MSV can also be demonstrated using microplates. By combining the 2 techniques, the replicating and MSV-rescuing abilities of MuLV stocks can be compared. In the initial study, complete correlation between the 2 functions was observed.

C. Infection of 3T3 cells with Rad LV did not result in any morphological alteration of the cells. However, a chronic infection was established, and 10^3 - 10^4 focus-inducing units of Rad LV, as measured in S+L- cells, could be recovered from infected culture fluids. Cloning of these Rad LV infected 3T3 cells indicated that each cell was capable of producing small amounts of infectious Rad LV.

D. The ability of MuLV to rescue infectious MSV from S+L- cells was inactivated by UV-irradiation at the same rate as the ability to form XC plaques or to induce lytic foci in S+L- cells. These results show that MuLV replication is required for the rescue of MSV from S+L- cells, consistent with the idea that MSV is "defective" at a very late stage in its replicative cycle, possibly involving virus packaging or coat formation. The ability of MuLV to form XC plaques or S+L- foci was the same at all levels of inactivation by UV, indicating that foci in each system are the result of virus replication.

Significance to Biomedical Research and the Program of the Institute:

A. A study of the nature of the interaction between feline sarcoma and leukemia viruses is of special interest since these agents can readily infect heterologous species including man. The Crandell cell line offers several practical advantages compared with primary FEF in a study of these viruses in the same way that the 3T3 mouse cell line has facilitated studies with the murine group.

B. The development of virus assay techniques using microplates is of potential value for cloning viruses as well as for the study of products of single virus-cell interactions.

C. Studies of the "defectiveness" of Rad LV and the influence of this virus on host control mechanisms may provide information relating to the nature of defectiveness of possible human leukemia viruses.

D. Stepwise inactivation of different virus functions is a first step towards a genetic analysis of oncogenic viruses and may provide the basis for a better understanding of the nature and mechanism of viral oncogenesis.

Proposed Course:

A. Attempts are being made to isolate nonproducing cells from CCC line infected with MSV(FeLV) using monolayers and agar suspension techniques.

B. Microplates are being used to clone leukemia viruses and to select mutants of both MSV and MuLV.

C. An analysis of the interactions of Rad-LV with 3T3 cells is in progress.

D. Further studies on the nature of the defectiveness of MSV and the role of MuLV in MSV replication are in progress.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4840
1. Viral Leukemia and Lymphoma
Branch, OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Non-Human Primate Surgery - Development of Special Techniques and Procedures in Addition to Routine Surgery

Previous Serial Numbers: None

Principal Investigator: Dr. Sergio A. Leiseca

Cooperating Units: Inside NIH
Dr. Roy F. Kinard, VLLB, NCI
Dr. Paul H. Levine, VLLB, NCI
Dr. Robert A. Manaker, VB, NCI
Dr. Jack Gruber, VB, NCI
Dr. Mary A. Fink, NCI

Outside NIH
Bionetics Research Laboratories
Kensington, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To provide to the National Cancer Institute, and other cooperating Institutes, embryonic and fetal material from the primate colony maintained at Bionetics Research Laboratories (BRL).

2. To provide surgical procedures required to maintain and improve the health of primate colony inhabitants.

Methods Employed and Major Findings:

1. C-sections provide most of the infants for the SVCP virus inoculation program at the BRL project.

2. C-sections are also used to study the effect of carcinogenic materials on fetuses inoculated in utero. Also, this procedure is used in amniocentesis for recovery of drugs in the amniotic fluids.

3. Developed and applied procedures for cannulation of thoracic lymph ducts in chimpanzees and rhesus monkeys for the continuous collection of lymph.

4. Developed skin graft procedures for use in radiation studies on rhesus monkeys.

5. Performed routine splenectomies on animals used in combined cancer-malaria studies, and on animals used in separate studies.

6. The C-section technique is also used to obtain fetal material for large amounts of primary cell cultures used in NCI and its collaborating laboratories.

7. Many operative procedures are done for various clinical conditions, complications, and accidents in the primate colony.

Significance to Biomedical Research and the Program of the Institute: Non-human primates have proven very useful in research on human viral diseases. They are man's closest phylogenetic relative and are the most desirable model system in research projects which cannot utilize humans directly. Much of the work in primate cancer research is dependent on the highest quality surgical procedures for the provision of sterile fetal tissues and healthy young free of pathogenic organisms. Surgical intervention for lymph and other tissues requires great professional skill for maximum animal health and comfort in addition to experimental validity.

Proposed Course: Continue to provide the best possible surgical procedures for primate cancer research.

Publications:

Sibinovic, S., Valerio, D. A., Landon, J. C., and Leiseca, S.: Maintenance of juvenile simians for oncogenic studies. Medica Primatologica. 2nd Conf. on Exper. Med. In press.

Serial No. NCI-4841
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Purification and Characterization of Polymerases from RNA Tumor Viruses and from Cells

Previous Serial Number: None

Principal Investigator: Dr. Jeffrey Ross

Other Investigators: Dr. Stuart A. Aaronson
Dr. Edward M. Scolnick
Dr. George J. Todaro

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To purify and characterize viral and cellular polymerases in order to study the mechanics of viral replication and transformation.

Methods Employed:

Standard biochemical purification and analytical techniques.

Major Findings:

1. Techniques for the solubilization and partial purification of the RNA-dependent DNA polymerase of Rauscher leukemia virus have been developed. The viral enzyme has been partially purified by column chromatographic procedures, and has a molecular weight of less than 100,000.

2. Enzymes capable of polymerizing deoxynucleoside triphosphates in the presence of synthetic templates have been partially purified from normal and leukemia virus-infected mouse cells. The template specificities, cation requirements, and chromatographic properties of these enzymes are being studied, in order to determine the relationships, if any, between these enzyme activities and the viral polymerase.

Significance to Biomedical Research and the Program of the Institute:

A better understanding of the relationships between viral tumor virus and cellular polymerases is potentially of great importance in the search for evidence of a viral etiology to human cancer.

Proposed Course:

Follow the leads in the program developed during the past year and to extend them with a goal of proving a viral etiology of human cancer.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4842
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The biochemistry and biophysics of leukemia and sarcoma viruses

Previous Serial Number: NCI-4836

Principal Investigator: Dr. Leo Phillips

Other Investigators:
Dr. Robert H. Bassin, VLLB, NCI
Dr. Peter J. Fischinger, VLLB, NCI
Dr. Daniel K. Haapala, VLLB, NCI
Dr. Shigeko Nomura, VLLB, NCI
Dr. Paul T. Peebles, VLLB, NCI

Cooperating Units: Inside NIH:
Electron Microscopy Section, VBB, NCI

Outside NIH:
None

Man Years:

Total:	2.00
Professional:	1.00
Others:	1.00

Project Description

Objectives:

These studies are concerned with the biochemical and biophysical properties of oncorna viruses. Our objectives are as follows:

1. To characterize the RNA's and the structural polypiptides of purified virions released from cells which have been transformed by sarcoma viruses.

2. To isolate and characterize the viral specific RNA's (ss - RNA, RF, and RI) and polypeptides of cells infected with leukemia and sarcoma viruses.

3. To determine which of the viral specific polypeptides is the RNA-dependent-DNA polymerase of the virions.

4. To determine what makes a sarcoma virus either competent or defective.

5. To determine the state of the sarcoma genome in S+L- cells which are not releasing particles; determine whether the genome exists as a plasmid, an episome, or whether it is integrated into the cellular DNA.

6. The ultimate goal of my research is to determine what makes one cell line permissive to oncogenic virus infection and another cell line nonpermissive. To infect by these viruses, therefore, an *in vitro* protein synthesizing system will be developed to answer this vital question.

Methods Employed:

The focus assay for sarcoma viruses and the helper assay for leukemia viruses are employed for the quantitation of the viruses. [^{3}H]-uridine incorporation in particles with a density of 1.16 g/cm^3, RNA-dependent-DNA-polymerase assays on supernatant fluids and electron microscopy are utilized to determine if virus is released from transformed cells. For characterization of the viral RNA's and polypeptides, standard biochemical and biophysical techniques (analytical and preparative ultracentrifugation, column chromatography, isotopic labeling, polyacrylamide gel electrophoresis, peptide fingerprinting, electrofocusing, *in vitro* protein synthesis, enzymology, etc.) are being employed.

Major Findings:

When mouse cells are transformed by MSV, some of the infected cells become "nonproducers", those which do not release any detectable virus, and some become "producers", those cells which release virus. The amount of virus released by "producers" varies as indicated by [^{3}H]-uridine incorporation at a density of 1.160 g/cm^3 and electron microscopy. Most of the sarcoma virions, which are released from "producers" have a suboptimal amount of RNA-dependent-DNA polymerase in comparison to leukemia virions. The RNA genomes of the sarcoma virions released from nonproducers have $S_{20}w$ values which are indicative of the complete genome (65S) to only a fraction of the genome (17S) unlike the leukemia virions which appear to have the complete genome (60-70S). The variation in the size of the genome of sarcoma virions could possibly account for their competency or noncompetency.

Significance to Biomedical Research and the Program of the Institute:

These biochemical and biophysical studies of oncorna viruses contain three avenues for obtaining pertinent knowledge for the possible cure of cancer: (1) studies which are being conducted on the viral RNA's and structural polypeptides could possibly determine if their mode of replication is by means of transcriptional-translational completes, and if so, it may be possible to effect a cure for cancer by specifically blocking the replication of these oncorna viruses either at the transcriptional or translational level; (2) studies which are being done to determine the disposition of the viral genome in sarcoma transformed, "nonproducer" cells could possibly enable the excision of the viral genome with interfering with the cellular genome; (3) studies which are being conducted to ascertain the property which makes one cell line permissive, while another cell line is nonpermissive, to infection by oncogenic viruses will be invaluable in effecting a cure for cancer.

Proposed Course:

These critical biochemical and biophysical studies of oncorna viruses will be continued in order of their complexity. In addition, *in vitro* protein synthesis studies will be initiated.

Honors and Awards:

Participated in the Cold Spring Harbor Tumor Virus Workshop, July 31 to August 23, 1970.

Publications:

Kramer, M. J., Bassin, R. H., Phillips, L. A., and Haapala, D. K.: Ultrastructural and biochemical evidence for virus production by murine sarcoma virus transformed mouse cells which are negative for leukemia virus. *Bacteriol. Proc*. In press.

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, Shigeko, and Fischinger, P. J.: Transformation of mouse 3T3 cells by murine sarcoma virus: release of virus-like particles in the absence of replicating murine leukemia helper virus. *Proc. Nat'l. Acad. Sci.*, U.S. In press.

Serial No. NCI-4843
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: A. Virus activation in chronic allogeneic disease in autoimmune mice

B. Studies with Rauscher leukemia virus propagated in human cell cultures.

C. Inhibition of Rauscher disease splenomegaly by dichloroacetylene

D. Induction of and sensitivity to interferon by cytomegalovirus in vitro

Previous Serial Numbers: 4888, 4889, 4891, 4897

Principal Investigator: Dr. John M. Easton

Other Investigators: Dr. Adi Gazdar, VLLB, NCI
Dr. Paul Peebles, VLLB, NCI
Dr. Dharam Ablashi, VLLB, NCI
Dr. Willie Turner, VBB, NCI

Cooperating Units: Inside NIH
Dr. Samuel Baron, LBV, NIAID
Dr. Herbert Oie, LBV, NIAID

Outside NIH
Lt. Charles Rose,USN, Toxicology Unit, National Naval Medical Center
Dr. J. Siegel, Toxicology Unit, National Naval Medical Center.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Serial No. NCI-4843

Project Description

Objectives:

A. Hirsch, et. al., have found that leukemia viruses rapidly become activated in young adult (BALB/c x A/J) F_1 hybrid mice inoculated with parental BALB/c spleen lymphoid cells, when both the F_1 and the parent have no detectable leukemia viruses. This project will first try to reproduce the work of Hirsch, et.al., using another F_1-parent combination, and then the technique will be used in an attempt to activate C-type particles in mouse strains associated with a high incidence of autoimmune disease.

B. A lot of Rauscher leukemia virus prepared at Hazleton Laboratories in BALB/c mice (RLV-HL) was found to infect human cell cultures by Wright, et. al. Studies are being performed to characterize this virus biologically; these involve induction of splenomegaly and interference with infection initiated by RLV.

C. Dichloroacetylene (DCA) is an extremely toxic gas sometimes found in systems having closed atmospheres for prolonged periods. A recent observation has suggested that inoculation of mice with Rauscher leukemia virus (RPV), with subsequent exposure of the animals to an atmosphere containing 1 part per million of DCA, will result in the inhibition of RPV splenomegaly. The proximate objective of these experiments will be to run controlled studies to see if there is a valid effect of the gas on the disease and on viral replication. Pathologic studies will be performed on the experimental animals to further study the inhibition and possible interaction between the virus and gas.

D. To determine the induction of and sensitivity to mouse cytomegalovirus (MCMV) and human cytomegalovirus (HCMV) *in vitro*.

Methods Employed:

A. Graft-virus-host reactions are induced by inoculating parental spleen cells into F_1 hybrids. C-type particles are detected using (S+L-) cells.

B. RLV grown on human cells is inoculated into newborn and weanling mice in the presence and absence of DEAE dextran. Routine necropsies are performed and spleen and body weights are measured. Virus is to be assayed by the XC test and also using (S+L-) cells. Relative infectivity for human and murine cells is determined by infectivity studies utilizing respective tissue cultures.

C. Experimental animals are maintained in sealed chambers in an atmosphere containing $1:10^6$ parts of DCA. Routine necropsies, including spleen and body weights, will be performed on experimental and control animals. RPV will be assayed using (S+L-) cells.

D. Mouse and human tissue culture cells are used for the replication of their respective cytomegaloviruses. Mouse and human interferon are prepared and titrated by standard methods.

Major Findings:

A.,B.,C. Experiments are in too preliminary a state to report any significant findings.

D. Results suggest that small but detectable amounts of interferon are produced by MCMV infected mouse embryo cultures. *In vitro* sensitivity studies have suggested that small doses of MCMV and HCMV manifest high sensitivity to the antiviral effect of interferon in cell cultures, whereas, higher doses of virus show a marked decrease in sensitivity to interferon. The presence of a small interferon-resistant fraction of virus-infected cells may account for the observations.

Significance to Biomedical Research and the Program of the Institute:

A. Activation of C-type viruses in mice with autoimmune disease would be of interest to determine the relationship between the two. It would also be of interest to determine if these C-type particles are oncogenic should methods be obtained to induce their replication.

B. The study of Rauscher virus growing in human cells could serve as a useful model for putative C-type particles involved in human neoplasia.

C. These experiments could lead to the development of a number of compounds useful in the treatment of neoplasms induced by viruses.

D. Cytomegaloviruses are closely related to such viruses as Herpesvirus saimiri and Ebstein-Barr virus and are thus worthy of having their properties investigated.

Proposed Course:

A., B., C. Experiments will be performed as described in preceding sections and elaborated upon as required.

D. Further experiments will be conducted in order to confirm and amplify the above results.

Honors and Awards:

None

Publications:

Easton, J. M., Rabson, A. S. and Malmgren, R. A.: Transformation of hamster cells *in vivo* and *in vitro* by an adenovirus 4-SV40 hybrid. *Int. J. Cancer* 5: 15-20, 1970

Serial No. NCI-4844
1. Viral Leukemia and Lymphoma Branch
OASDVO, Etiology
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Quantitation of Feline Leukemia and Sarcoma Viruses and Their Specific Antibodies

Previous Serial Number: None

Principal Investigator: Dr. Kyu M. Lee

Other Investigators: Dr. Louis R. Sibal
Dr. Mary A. Fink
Dr. Vincent W. Hollis, Jr.
Dr. Ernest J. Plata

Cooperating Units: Inside NIH
Dr. Robert H. Bassin, VLLB, NCI
Dr. Shigeko Nomura, VLLB, NCI
Dr. Peter J. Fischinger, VLLB, NCI

Outside NIH
Dr. Fernando Noronha, Ithaca, New York

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description

Objectives:

The principal objectives of this project are to improve, replace or add to the methods presently available for *in vitro* infectivity assay of feline leukemia and sarcoma viruses as well as for the detection and measurement of antibodies specific to these viruses. To achieve this goal, attempts are made:

1. To develop a focus-reduction-neutralization test for feline leukemia virus and to correlate the results with those obtained by other methods such as passive hemagglutination and immunodiffusion.

2. To test a wide variety of homologous as well as heterologous cell culture systems in a search for cell lines suitable for in vitro focus assay.

3. To prepare a new viral pseudotype for use in cross-focus-reduction-neutralization tests.

4. To develop colorimetric and colorimetric-neutralization tests for feline sarcoma virus.

5. To develop the conglutinating complement absorption test for use with cat sera.

6. To obtain feline analogy of avian "nonproducing" (NP) or murine "sarcoma-positive leukemia-negative" (S^+L^-) cell lines.

Methods Employed:

In order to eliminate anticomplementary activity from feline leukemia antigen to be used in the conglutinating complement absorption test, the density-gradient-purified and ether-treated antigens were passaged through Sephadex G-150 columns (Dr. Hollis, NCI-4827). The pooled antigen-containing fractions proved to be less anticomplementary than the original.

Major Findings:

1. Cat serums known to be positive by passive hemagglutination tests and the standard goat antiserum to feline leukemia virus (Rickard isolate) were tested for their ability to reduce focus formation. The indicator virus was a feline leukemia virus pseudotype of a murine sarcoma virus, MSV (FeLV), prepared by Fischinger and O'Connor. The serum reduced by twofold to tenfold the number of foci produced by the indicator virus as compared with the controls. In general, the degree of focus formation correlated well with the HA titer.

2. A feline kidney cell line (6CC) established by Crandell was compared with feline embryonic fibroblast (FEF) cells as to their response to focus formation by MSV (FeLV); 6CC appeared to be nearly as susceptible to the infection of MSV (FeLV) as FEF, virus titers on 6CC being about one half log lower than those on FEF; coinfection of 6CC as well as FEF cultures with FeLV and defective stock of MSV (FeLV) induced increased number of foci, and converted the two-hit titration curve of the defective indicator virus to a one-hit pattern; pretreatment of both 6CC and FEF cells with DEAE-Dextran enhanced by 3- to 4-fold focus formation by MSV (FeLV) either alone or with exogenous FeLV; a preparation of the Gardner-Arnstein strain of feline sarcoma virus (FeSV-GA) exhibited "helper" activity for a defective stock of MSV (FeLV), and it was possible to use this procedure for quantitation of the FeSV-GA on 6 CC.

Propagation of MSV (FeLV) in many species of cells including dog, cattle and dolphin cells resulted in morphologic alteration and on coinfection with FeLV focus formation was augmented, indicating that FeLV per se could infect the same cells. The standard goat antiserum failed to reduce significantly the number of foci produced in dolphin cultures by FeSV-SA, indicating coat antigens of FeLV and FeSV-GA are not identical.

3. Fischinger and O'Connor originally prepared MSV (FeLV) by infecting cat cells with an interviral aggregate of defective MSV and a Rickard isolate of FeLV followed by serial passage of the progeny virus in cat cells. The virus was considered to contain the MSV genome within an envelope specified by FeLV. This investigator has shown that coinfection of MSV (FeLV) with FeSV-GA not only enhances focus formation by defective stock of MSV (FeLV) but converts the two-hit titration pattern of MSV (FeLV) to one-hit, indicating that the active constituents in the FeSV-GA stock behave as "helper" virus to defective MSV (FeLV). It would then be predicted that if cultures are infected with the defective MSV (FeLV), diluted beyond the end point for focus formation, and then superinfected with the FeSV-GA, the resulting progeny MSV particles should acquire coat proteins of the FeSV-GA. In accordance with this hypothesis, a virus stock was prepared which was considered to contain a new pseudotype, MSV (FeSV-GA). The results of direct focus-reduction-neutralization tests by using the putative MSV (FeSV-GA) and the original MSV (FeLV) against the standard goat antiserum to FeLV are shown in the Table. The finding is interpreted to indicate that coat antigens of FeLV and FeSV-GA are related but not identical.

Table. Comparative Focus-Reduction-Neutralization Tests With Modified MSV Pseudotypes Against Anti-FeLV Goat Serum

Anti-FeLV goat serum, final dilution	Percentage focus-reduction	
	Virus I*	Virus II**
1:4	100	90
1:8	96	7
1:16	76	5
1:32	52	5
1:64	30	5
1:128	5	5
Control	0	0

*Virus I - MSV (FeLV)
**Virus II - Putative MSV (FeSV-GA)

4. There are difficulties associated with quantitating the FeSV-GA because of the facts that the efficiency of focus formation is low; virus stocks, even at the highest concentration available, fail to produce foci in all inoculated cultures; and focus-forming activity is rapidly lost upon dilution. It was found, however, that infection of FeSV-GA, even at a dilution as high as 10^{-6} markedly acidify bovine embryonic spleen cell cultures, and the acid production could be used as a simple criterion for the presence of virus. On this basis, an expedient colorimetric-neutralization test has been developed.

5. Certain cat sera are known to be pro-complementary in the sense that they contain heat-stable hemolytic fractions which behave as complement in the hemolytic system, and therefore, renders them unsuitable in the complement-fixation test. However, it is known that cat sera will fix horse complement, which is nonhemolytic, in the presence of specific antigen in the conglutinating complement absorption system. This suggests that conclusions should not be drawn on the basis of complement-fixation tests alone on cat sera. Preliminary studies indicate that the conglutinating complement absorption test may be applicable for the detection of antibodies to feline leukemia virus and its microtest could be developed.

6. In cooperation with Dr. R. Bassin attempts are made to obtain NP or S^+L^- cell lines of feline origin. We have found that 6CC cat kidney cells (Crandell) fail to form colonies in semisolid agar prior to infection with MSV (FeLV) but did so after infection. We were successful in isolating colonies transformed by MSV (FeLV), and the resulting lines are being studied. Preliminary observations seemed to indicate that when a preparation of MSV (FeLV) which gave two-hit titration curves in monolayers of 6CC cell cultures was employed, the number of colonies appearing in semisolid agar at optimal cell concentrations was directly proportional to the concentration of MSV (FeLV).

Significance to Biomedical Research and the Program of the Institute:

The availability of diversified methods for virological and serological assessment of feline leukemia and sarcoma should markedly improve our understanding of the diseases and their causative agents. For instance, there is a need for a method by which a large number of serum samples can be tested. A colorimetric-neutralization test for FeSV could be a reliable, sensitive seroepidemiologic tool with which one might be able to test sufficient sera in order to determine the involvement, if any, of nonfeline species, including man, in the natural history of the feline tumor viruses. For the cat the conglutinating complement absorption test should be preferred to the complement fixation test because of procomplementary activity of certain feline sera. Finally, the antigenic properties of the feline leukemia and sarcoma viruses are not well defined; the use of cross-neutralization tests with viral hybrids may aid in elucidating possible strain differences for these viruses.

Serial No. NCI-4844

Proposed Course:

The project has ceased.

Honors and Awards:

None.

Publications:

Sibal, L.R., Fink, M.S., Plata, E.J., Kohler, B.E., Noronha, F. and Lee, K.M.: Methods for the detection of viral antigen and antibody to a feline leukemia virus (a preliminary report). J. Nat. Cancer Inst. 45: 607-612, 1970.

Lee, K.M.: Comments on feline leukemia virus. In Proc. of the Colloquium on Selected Feline Infectious Diseases. J.A.V.M.A., 1971. In press.

SUMMARY REPORT

VIRAL BIOLOGY BRANCH

July 1, 1970 - June 30, 1971

The Viral Biology Branch conducts research to develop and apply new conceptual and technical approaches to the study of the viral etiology and control of neoplastic diseases. More specifically, the Viral Biology Branch is active in: The isolation and characterization of various constituents of tissues which are involved in or related to the carcinogenic process; the study of the biological interactions of viruses with host cells in vitro and in vivo; the investigation of viruses in relation to human neoplasia by virological, immunological and biochemical methods; the study of interrelationships between viruses in the neoplastic process; the study of cells and viruses at the ultrastructural level; the investigation of host factors in relation to development of neoplastic diseases; and the control of neoplasia in experimental animals.

The Cell Biology Section studies methods for increasing the antigenicity of weak tumor transplantation antigens. Vaccination of mice with a homogenate of tumor cells previously infected with influenza virus gave significant protection of the animals against challenge with viable cells of that tumor. No protective effect was observed in control animals vaccinated with homogenates of the tumor cells not infected with influenza virus or with homogenates of normal cells. The presence of the influenza virus antigen on tumor cells increased the immunogenicity of tumor cell antigens.

Methods for quantitatively measuring contact inhibition of locomotion were developed and successfully applied to normal and SV40 transformed cells.

Evidence was obtained demonstrating that the so-called membrane antigen detected in EB virus-infected cells was not integrated into cell membranes. This antigen is loosely adsorbed to either the inner or outer aspect of cell plasma membranes, and may represent non-specifically adsorbed viral components.

The Head of the Cell Biology Section serves as Project Officer on six extramural research contracts within the Special Virus Cancer Program and is a working member of the Immunology Segment.

The Virus and Disease Modification Section conducts a multi-disciplinary approach to the detection, prevention, treatment and/or control of leukemia and sarcoma. Virus infection and chemical treatments were used in attempts to modify tumor cell surface antigens. For this prupose, an AKR mouse line carrying Gross virus was infected with several non-oncogenic viruses. Newcastle disease virus infection induced loss of the Gross leukemia virus antigen complex in half of the cells, while measles and Germiston virus infection resulted in the loss of G+ cellular antigens and gs-1 antigen from over 90 percent of the cells. AKR cells infected with Germiston virus lost their capability to grow as tumors.

In animals previously vaccinated with BCG, a mixture of BCG and Moloney pseudotype mouse sarcoma virus (MSV-M) induced no tumors after inoculation although a similar inoculum of MSV-M in non-BCG-treated mice resulted in tumors in all infected animals. The protective effect of adoptive immunotherapy was demonstrated in mice bearing the transplantable leukemia, LSTRA. Tumor-bearing mice were placed in remission with bis-chloronitrourea (BCNU), and then inoculated with tumor-immune mouse spleen cells. After 70 days observation 94 percent of the experimental animals survived in comparison with 40 percent survivors among animals treated with BCNU alone. Untreated mice inoculated with LSTRA cells succumb in 14 days.

Interferon therapy was effectively applied in mice infected with the Moloney sarcoma virus plasma variant (MSV-PV). The survival rate of virus-infected mice treated with the interferon-inducing poly Ir:Cr-poly-D-lysine complex was nearly twice that achieved with poly Ir:Cr alone. Mice immunized with formalinized MSV-PV were also refractory to challenge with MSV-M and Friend leukemia virus as well as with MSV-PV.

A micro assay developed for delta-aminolevulinic acid (ALA) synthetase, an important enzyme in the biosynthesis of red cell porphyrins, was applied to detect enzyme activity in leukemic white cells. ALA synthetase was found in leukocytes from patients with acute and chronic myelogenous leukemia and acute monocytic leukemia, but not in leukocytes from donors with chronic lymphocytic leukemia nor from normal donors. These observations may have diagnostic value. Further studies were made with IM-1 cells, a line of cultured lymphoblasts which have a completely functional heme biosynthetic pathway. ALA synthetase was detected in these cells and found to be sensitive to puromycin but not to actinomycin D. This suggests a shorter half-life for the enzyme than for its messenger RNA. Polyphoric fibroblasts were sensitive to rotenone, an inhibitor which reduces oxygen consumption by blocking transfer of reducing equivalents between NADH and cytochrome B. These investigations on heme synthesis in leukocytes may reveal the importance of porphyrin synthesis in the leukemic state.

The Head of the Virus and Disease Modification Section serves as Chairman of the Special Animal Leukemia Ecology Segment, SVCP, and as Project or Assistant Project Officer on eight research contracts. Other investigators within the Section also have Project Officer duties.

The Human Tumor Studies Section conducted studies on EB virus. In an attempt to select a lower primate susceptible to infection by EB virus, different species of monkeys were first screened for antibodies reactive with EB virus. Immunofluorescence tests showed a high incidence of antibodies in all Old World monkeys obtained from the wild but not in New World monkeys. Monkey sera reacted with the Beta antigen in EB virus-infected cells and gave a line of identity with human sera in immunodiffusion tests. Seronegative monkeys were inoculated with infectious EB virus. Attempts to establish lymphoblastoid cell cultures from infected animals were not successful.

Specimens of tissue from various organs of Burkitt tumor patients taken post-mortem were tested for the presence of EB virus antigens. In one case, immunodiffusion tests showed viral antigen in the spleen and tumor tissue but not in other organs. Other case material was negative.

The Mason-Pfizer monkey virus (M-PMV), recovered from a spontaneous rhesus monkey mammary tumor, banded at a density of 1.166 gr/cc on density gradient centrifugation. The RNA of the virus analyzed by polyacrylamide gel electrophoresis appeared to be of the same size as other RNA tumor viruses. Its calculated molecular weight was 7.4×10^6 daltons. Applying the Spirin formula yielded an estimated sedimentation value of 56 S,which does not agree with reported S_{20w} values of 65-70 S which were calculated from sedimentation velocity data. The basis for this variation is under investigation.

Hemadsorption tests made on M-PMV infected cells showed no evidence of the presence of SV5 virus. Preliminary serology yielded no reaction between antiserum to M-PMV and simian foamy virus types 1,2,3,4,5,6, or 7.

The Head of the Human Tumor Studies Section is Project or Associate Project Officer on six research contracts within the Special Virus Cancer Program.

Within the Microbiology Section, a study was completed *in vitro* on a viral inhibitor reported to be present in the JLS-V5 cell line chronically infected with Rauscher virus. Silicotungstic acid, a protein precipitant applied in the preparation of this inhibitor, was found to be the active factor inhibiting replication of murine tumor viruses *in vitro*. No activity was found by another laboratory testing the inhibitor *in vivo*.

The lactic dehydrogenase virus (LDV) was found to potentiate oncogenesis by murine sarcoma viruses of the Harvey (MSV-H) and Moloney (MSV-M) strains. Co-infection of mice by LDV and MSV-H gave a 90 percent increase in tumor incidence and mortality. The influence of an inocuous agent such as LDV on the incidence of disease induced by a tumor virus is particularly significant.

A complex of poly-D-lysine and poly Ir:Cr induces higher levels of interferon *in vivo* than does poly Ir:Cr alone. When serum interferon responses were determined in different strains of mice, activity varied by a factor of about 100 from the most to the least responsive mouse strain.

The Electron Microscopy Section is newly established. Collaborative studies with other investigators were begun and support services for the Viral Oncology Area were instituted. A considerable amount of routine examination of tissues and cell cultures to detect virus was completed. Several of the more recent murine sarcoma and lymphoma virus isolates were studied in the organs of infected animals of different species. A simian cytomegalovirus isolate was tentatively identified on the basis of morphological criteria, and identity was later confirmed serologically. Studies with Herpesvirus saimiri to determine its host range were monitored.

The possibility that M-PMV might be a simian foamy virus was investigated by comparison with seven different types of known foamy viruses in human kidney cell cultures. Unlike M-PMV, all the foamy viruses induced cellular degenerative changes. Although foamy viruses form intracytoplasmic A-type particles which bud from the cell surface as does M-PMV, the mature extracellular particles differ in that they form spiked particles. Further, antiserum to M-PMV failed to neutralize the foamy agents.

Biopsies of a spontaneous mammary carcinoma of a Siamese cat contained 100 μm diameter particles resembling virus. The viral nature of these particles is still uncertain.

Research conducted within the Virus Studies Section was discontinued upon the retirement of the principal investigator, Dr. Stewart. Under this project, four human tumor cell lines spontaneously transformed without morphological evidence of the presence of virus. Transmission studies with clarified and with filtered fluids from these cultures were suggestive of the presence of a factor inducing transformation of other cells.

The Office of the Chief coordinates the research efforts of investigators in each of the above sections and collaborative studies with investigators in other laboratories. The Chief serves as Chairman of the Developmental Research Segment of the Special Virus Cancer Program and is responsible for the administration and management of the extramural research contracts within the Segment. He is supported by the Vice-Chairman of the Segment, secretarial assistance, and investigators within and outside the Branch who serve as Project Officers and/or members of the working contract review group.

Proposed Course of Effort Within the Viral Biology Branch:

Investigations within the newly reorganized Branch have been largely directed to the characterization of specific viruses immunologically and biochemically, to the evaluation of measures for control of virus-induced neoplasia, to the study of normal and malignant cell *in vitro*, and to the initiation of intensive investigations on cell-mediated immune mechanisms. These studies will continue.

A susceptible lower primate for studies on the EB virus will be sought. Present evidence indicated that a number of species of monkey are naturally infected with a virus antigenically related to EB virus. Attempts will be made to establish infection in these species as well as in species of New World monkeys.

The tumor promoting effect of non-oncogenic viruses interacting with oncogenic strains is highly significant in relation to human neoplasia. Investigations will continue to determine the mechanism for such potentiating activity.

Immunological control measures effective against virus tumor development will be further evaluated. Methods to increase the antigenicity of weakly antigenic tumors will be sought. Studies will be conducted to ascertain the nature of factors effectively transmitting specific cellular immunity within and across species.

Biochemical characterization of specific viruses will be continued.

Publications and Other Activities:

During the reporting period a total of 15 papers were published or accepted for publication. Members of the Branch presented by invitation more than twelve lectures to research groups in this country and abroad.

In addition to the intramural research efforts of members of this Branch, most also contribute substantial amounts of their time to support research contract projects within the Special Virus Cancer Program. Specifically, Dr. Michael Chirigos, Associate Chief of the Viral Biology Branch, serves as Chairman of the Special Animal Leukemia Ecology Segment and Dr. George Burton is Executive Secretary of this Segment. Dr. Jack Gruber is the Vice Chairman and acts as Executive Secretary for the Developmental Research Segment, Dr. Timothy O'Connor serves as advisor in Molecular Virology to the Associate Scientific Director for Viral Oncology and as a working member of the Developmental Research Segment Contract Review Group. Dr. Charles Boone is a working member of the Immunology Segment Contract Review Group. In addition, these investigators, as well as other investigators within the Branch, act as Project Officers on research contracts within the SVCP. These duties require direction and consultation with contractors, site visits, preparation of regular reports, and attendance and presentation of data during contract reviews.

Serial No. NCI-4874
1. Viral Biology Branch
OASDVO, Etiology
2. Electron Microscopy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ultrastructural Studies on Tissue and Viruses in Relation to Neoplastic Disease

Previous Serial Number: None

Principal Investigator: Dr. Harish C. Chopra

Other Investigators: Dr. Michael J. Kramer
Mr. Bolivar J. Lloyd
Mrs. Elizabeth Mitchell

Cooperating Units: Dr. D. Ablashi, VLLB, NCI (NCI-4834)
Dr. E. Plata, VLLB, NCI (NCI-4828)
Dr. A. Gazdar, VLLB, NCI (NCI-4824)
Dr. R. Bassin, VLLB, NCI (NCI-4823)
Dr. V. Dunkel, VBB, NCI (NCI-4881)
Dr. S. Margolis, VBB, NCI (NCI-4882)
Dr. W. Woods, VBB, NCI (NCI-4877)
Dr. A. Hellman, BCC, OASDVO, NCI (NCI-4805)
Dr. J. Gibbs, C&FR, NINDS
Dr. J. Hooks, C&FR, NINDS

Man Years:

Total:	7.3
Professional:	3.4
Other:	3.9

Project Description

Objectives:

To conduct ultrastructural studies on viruses and tissues of significance in neoplastic processes; to conduct collaborative studies with other investigators on problems of mutual interest; and to provide a supportive function in electron microscopy to investigators within the Viral Oncology Area.

Methods Employed:

Tissues, cultured cells, and virus concentrates were prepared by thin sectioning and negative staining techniques and examined by electron microscopy. Observations were interpreted, evaluated, and suitably recorded.

Major Findings:

The electron microscopy laboratory was reorganized and collaborative studies with other investigators were initiated.

In collaboration with Dr. W. Woods, specimens of tissues and cultured ascites cells from a lymphomatous dog were examined, but no virus particulates were detected. General electron microscope support was provided for Dr. Woods' studies on a number of murine neoplasms.

In collaboration with Drs. Dunkel and Margolis, fractionated components of EB virus-infected human cells were examined as part of a study on the intracellular localization of viral antigens. Further, cell cultures infected with virus isolated from a spontaneous tumor of the monkey were monitored for viral content to support characterization of the viral nucleic acid.

A solid lymphatic mouse sarcoma under investigation by Dr. Gazdar was found to contain virus of the C-type. The virus was found budding from lymphoblasts and was detected in the dura as well as in hematopoietic tissues of animals with tumor. In other collaborative work, a new strain of sarcoma virus from NZB/NZW F1 mice proved to be of the C-type. Virus was found in infected cultures of rat and mouse tissue. Tumors induced by the virus in hamsters also contained the virus particles.

Clones of 3T3 mouse cells transformed by Moloney mouse sarcoma virus which were free of leukemia virus were selected by Dr. Bassin. Examination showed the presence of only small numbers of virus particles with electron-lucent centers. Up to 100-fold increase in virus particles was detected following addition of leukemia virus.

Electron microscope support was given to Dr. Plata for a project to determine the relationship of viral immunity to Rauscher leukemia virus-induced tumor cell growth. The study showed a direct relationship between the capacity of the immune host to inhibit tumor cell growth and the phase of virus infection, percent of tumor cells with surface antigen, and the quantity of surface antigen.

A spontaneous lymphosarcoma recovered from Balb/C strain mice by Dr. Hellman contained both intracisternal A particles and budding C particles. Attempts will be made to determine the relationship between both types of particles.

Morphological studies of a virus isolated by Dr. Ablashi from oral and anal swabs of an owl monkey tentatively identified the agent as a simian cytomegalovirus. This was confirmed by follow-up serology. The virus observed in the cytomegalic cell inclusion body was composed of empty capsids and capsids with a central core. Nuclear filamentous material appeared to be incorporated into the virus capsids. Virus in the cytoplasm was enveloped during passage from the nucleus. Mature virus particles were often observed in cytoplasmic vacuoles, while the cell showed cytopathological changes characteristic of cytomegalovirus infection.

A comparative study of simian foamy viruses and the Mason-Pfizer monkey virus isolated from a spontaneous rhesus monkey mammary tumor was conducted in collaboration with Drs. Gibbs and Hooks of the Institute of Neurological Diseases and Stroke, NIH. Seven different types of known foamy agents were inoculated into human kidney cultures. Electron microscopy showed that, unlike the Mason-Pfizer virus, the foamy agents produced degenerative changes in the kidney cells. Although the foamy agents also form intracytoplasmic A-type particles which bud from the cell curface, the extracellular viruses differed from the C-type morphology of the Mason-Pfizer virus. They have surface spikes not observed in the Mason-Pfizer virus. Antiserum to the latter agent failed to neutralize the foamy agents.

Tumor biopsies of a Siamese cat with mammary carcinoma were obtained from the Mason Research Institute. Tissue samples and cultured tumor cells were examined in thin section and revealed the presence of a virus-like particle 100 µm in diameter with a central nucleoid. Further study is required to determine the viral nature of the particulates.

A human rhabdomyosarcoma and cultures of this sarcoma provided by Dr. Sarah Stewart revealed the presence of viral-like particles. The viral nature of these particles could not be definitively established owing to their small number and inability to grow in other cultured cells. Other human tumors examined contained no virus.

Significance to Biomedical Research and the Program of the Institute:

These collaborative ultrastructural investigations provide necessary support to specific research projects of interest to the program within the Viral Oncology area. The Electron Microscopy Section thus provides a service function for investigators and collaborates in greater depth on individual research projects.

Proposed Course:

This activity will be maintained.

Honors and Awards:

None.

Publications:

Rabstein, L. S., Gazdar, A. F., Chopra, H. C., and Abelson, H. T.: The early morphological changes associated with infection by a murine non-thymic tumor virus. *J. Nat. Caner Inst.*, in press.

Serial No. NCI-4875
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Control of Oncogenic Viruses and Induced Diseases Mediated by External Factors

Previous Serial Number: NCI-4828

Principal Investigator: Dr. Michael A. Chirigos

Other Investigators:
Dr. Wilna Woods (NCI-4877)
Dr. Donald B. Schwartz (NCI-4879)
Dr. Paul Ebert (NCI-4876)
Dr. John Pearson (NCI-4878)
Dr. Willie Turner (NCI-4889)
Dr. Gary Pearson (NCI-4880)

Cooperating Units :

Inside NIH
Dr. Jack Gruber, VBB, NCI
Dr. Tom Cameron, CCNSC, NCI
Dr. Richard Adamson, LCP, NCI

Outside NIH:

Dr. G. Spahn, Microbiological Associates, Inc., Bethesda, Maryland
Dr. David Howell, Ft. Detrick, Frederick, Maryland
Dr. E. Jensen, Hazleton Laboratories, Falls Church, Virginia

Man Years:

Total: 5.0
Professional: 2.0
Other: 3.0

Project Description

Objectives:

The overall aim of this project is to determine the most effective means of controlling leukemias and sarcomas. Several therapeutic approaches are examined in animal model systems to assess the most feasible approach that can be employed which will exert maximum antitumor activity, with the least deleterious effect on the host. Host factors which may be affected by therapy are immuno-competence, organ and/or cell toxicity, kidney or liver damage.

Methods Employed:

A. Virus and Tumor Animal Systems.
The following are employed for *in vitro* and *in vivo* studies.
1. The Rauscher, Friend, Moloney, Graffi and Gross leukemogenic viruses, and the Moloney, Harvey, Kirsten and Plasma Variant sarcomas.
2. AKR mice with spontaneous lymphoma.
3. Dunning lymphoma.
B. Humoral and cellular antibodies as measured by standard procedures or new methods developed by the Section staff.
C. *In vitro* and *in vivo* assay systems for interferon developed by the section.

Major Findings:

1. Interferon. The complex formed with poly-D-lysine and poly(I)·poly(C) is far superior in induction of interferon than poly(I)·poly(C) alone. Interferon titers are increased 100-fold and are maintained in blood for longer periods of time (24 hours), in comparison to poly(I)·poly(C) where titers induced are lower and of short duration. The serum interferon responses of a series of inbred strains of mice inoculated with the complex decreased in the order: Swiss>DBA/2>C_3H>BALB/c>CF-1>AKR>C_{57}Bl/6>NAB>SJL>NZW. The poly(I)·poly(C)-poly-D-lysine complex has been employed in treatment of MSV(PV). A 27% greater effect was attained than when poly(I)·poly(C) was used alone.

2. Immunoenhancers. BCG (an attenuated strain of Mycobacterium bovis) has been used successfully in enhancing cellular immunity against MSV(M). Complete protection against a lethal challenge was achieved by combining BCG and MSV(M) in an initial inoculation. An effective synergistic effect was achieved against a transplantable Moloney leukemia (LSTRA). An 87% survival rate was attained in mice treated with drugs and subsequently with BCG.

3. Inhibitors. An extensive study was completed on a viral inhibitor reported to be present in the JLSV-5 cell culture. Silicotungstic acid (STA), the protein precipitant employed for isolating the inhibitor from cells, was found to be the active factor in inhibiting replication of Moloney leukemia virus *in vitro*. No *in vivo* effect of inhibitor or STA was demonstrated against Friend leukemia virus.

4. Virus potentiation. Lactic dehydrogenase virus (LDV) was found capable of potentiating Murine Sarcoma Virus Moloney and Harvey oncogenesis *in vivo*. Of particular significance was the observation that MSV-H which induces tumors in less than 10% of mice infected as adults was enhanced by co-infection by an inocuous agent such as LDV. A 90% increase in tumor incidence, infection, and death was attained in mice co-infected with LDV and MSV-H.

5. Delayed hypersensitivity. Immunological and delayed hypersensitivity

reactions are being investigated with murine, rat and canine tumor systems. Tumor cell lines are being employed for colony inhibition and Cr^{51} release studies on cell mediated immunity in appropriate tumor systems, as well as in antigenic modification studies. Virus infection of tumor cells and chemicals is being employed in attempts to modify tumor cell surface antigens.

6. Adoptive immunotherapy. Effective control of a transplantable leukemia (LSTRA) has been attained in mice by chemo-immuno-therapy. Mice with systemic leukemia were put into remission with bis-chloronitrousourea (BCNU) and the mice treated with immune spleen cells. This combined therapy resulted in a 94% survival rate, a 54% greater survival than observed in the drug treated group.

Significance to Biomedical Research and the Program of the Institute:

The multi-disciplinary approach represented in this project is almost entirely directed towards the prevention, treatment and control of cancer. It is necessary to work with animal systems to gain basic information which is ultimately applied to the control of human neoplasia. The greatest progress in control of human leukemia has been by drug therapy which has several serious limitations. A study of the animal model systems, described herein, revealed that immunotherapy is an excellent control measure either alone or in combination with drug therapy. Other immuno-therapeutic applications are worthy of further investigation, particularly the use of interferon. Therapeutic approaches are presently at hand, and if better understood and judicially applied, can serve to better control human cancer.

Proposed Course:

Each of the areas described under objectives will be pursued. More emphasis will be placed on refining the therapeutic measures already developed for the more efficient control of leukemia, lymphoma and sarcoma. Efforts will be made to extend the application of therapy to the existing animal systems, so that the information gained from such studies can be applied more rapidly and meaningfully in the clinic.

Further *in vivo* studies will be conducted with the murine leukemia-sarcoma complex of rodents, to determine or elucidate the mechanism of tumor induction by low virus input. Such studies may lead to a better understanding of the possible role of viral co-carcinogenesis in human leukemia and/or sarcoma.

Honors and Awards:

Dr. Chirigos presented, by invitation from the New Jersey Society for Medical Education and Research, a seminar on "Treatment and Control of Leukemia" at a conference held at Glassboro State College, Glassboro, New Jersey.

Dr. Chirigos presented, by invitation, a seminar on "Therapy of Leukemia" to American Veterinary Medical Association at Cornell University.

He presented, by invitation, a seminar on "Virus-Cancer Complex" to National Science Foundation Science Trainees at Western Maryland College, Westminster, Maryland.

Publications:

Pearson, J. W., Gibson, W. T. and Chirigos, M. A.: Use of a Murine Sarcoma Virus in an *In Vivo* Assay for Antiviral and Antitumor Agents. *Cancer Res*. 30: 2024-2028, 1970.

Spahn, G. J., Turner, W., Peters, R. L., and Chirigos, M. A.: Enhancement of Friend Leukemia Virus Infection in Mice by Guaroa Virus: Quantitation and Action of Various Oncogenic and Nononcogenic Viruses. *Applied Microbiology* 20: 551-554, 1970.

Woods, W. A., Wivel, N. A., Massicot, J. G. and Chirigos, M. A.: Characterization of a Rapidly Growing AKR Lymphoblastic Cell Line Maintaining Gross Antigens and Viral Replication. *Cancer Res*. 30: 2147-2155, 1970.

Turner, W., Gibson, W. and Chirigos, M. A.: Enhancement of Murine Sarcoma Virus (Moloney) Infection in Mice by Guaroa Virus. *Cancer Res*. 30: 2645-2651, 1970.

Woods, W. A., Massicot, J., and Chirigos, M. A.: An Immunofluorescent Focus Assay for Gross Leukemia Virus. *Proc. Soc. Exp. Biol. and Med*. 135: 772-777, 1970.

Ebert, P. S., Maestri, N. E. and Chirigos, M. A.: Erythropoietic Responses of Mice to Infection with Rauscher Leukemia Virus. *Cancer Res*. (in press).

Rice, J. M., Turner, W., Chirigos, M. A. and Spahn, G. Dose Responsiveness and Variation Among Inbred Strains of Mice in the Production of Interferon After Treatment with Poly(I)·Poly(C)-Polylysine Complexes. *J. of Bacteriology* (in press).

Chirigos, M. A.: Current Studies on the Therapy of Experimental Leukemia. *Comparative Leukemia Research* 1969 (in press).

Serial No. NCI-4876
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical Alterations Occurring in Host Tissue as a Result of Oncogenic Virus Infection and Tumor Induction

Previous Serial Number: NCI-4830

Principal Investigator: Dr. Paul S. Ebert

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. Willie Turner, VBB, NCI (NCI-4889)
Dr. Wilna Woods, VBB, NCI (NCI-4877)
Dr. John Pearson, VBB, NCI (NCI-4878)
Dr. V. Dunkel, VBB, NCI (NCI-4881)
Dr. T. O'Connor, VBB, NCI (NCI-4888)
Dr. E. Chan, VBB, NCI, Visiting Scientist
Dr. D. P. Tschudy, Metabolism Branch, NCI
Dr. H. L. Bonkowsky, Metabolism Branch, NCI
Dr. D. N. Buell, GL&C, NCI

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:
1. To further investigate the feasibility of developing the assay for δ-aminolevulinic acid (ALA) synthetase as a possible test for acute leukemia.
2. To investigate the characteristics of ALA synthetase in a continuous leukocyte line derived from a malignant lymphoma as a model for lymphoblastic leukemia.
3. To determine the significance of the heme biosynthetic pathway in leukocytes.
4. To verify the existence of a potent antiviral inhibitor found in the supernatant fluid of JLS-V5 tissue culture cells.

5. To elucidate the biochemical defect(s) in the heme pathway of patients afflicted with acute intermittent porphyria by studying the enzymology and cytochrome chain of cultured skin fibroblasts.

Methods Employed:
Conventional enzymatic and biochemical procedures were employed to study biochemical alterations occurring in normal and virus-infected tissues and tissue culture-grown cells. ^{14}C-ALA production in murine tissues and tissue culture cells was quantitatively measured by passage of homogenized and incubated tissue through Dowex 50 columns. Radioactivity in column eluates was determined in a liquid scintillation counter. Attempts were made to prepare a viral inhibitor by precipitating proteins in tissue culture media and isolating a soluble active factor by dialysis and lyophilization.

Major Findings:
1. Heme synthesis in leukocytes: Attempts were made to study the heme biosynthetic pathway in IM-1 cells, a lymphoblastoid cell line originated from a malignant lymphoma. A completely functional heme pathway was found in this cell line as shown by the incorporation of ^{14}C-glycine and ^{14}C-succinate into heme. Glycine and succinate are substrates for the control enzyme ALA synthetase, an inducible enzyme which is absent in mature human white blood cells. ^{14}C-ALA was also incorporated into heme, further suggesting that the heme pathway beyond the control step is intact in the IM-1 cell line. Characteristics of the control enzyme in this cell line were studied utilizing a recently developed radiochemical micro assay. The enzyme does not appear to be inducible by compounds such as allylisopropylacetamide, etiocholanolone, progesterone, or erythropoietin, which can induce the enzyme in spleen or liver. ALA synthetase activity was almost twice as great during the log phase of cell growth as it was during the stationary phase. The enzyme is sensitive to puromycin, but not to actinomycin D, suggesting a shorter half-life of the enzyme than its messenger RNA. In rat liver the half-life of messenger RNA for the enzyme and ALA synthetase are each about 1 hour.
2. Potentiation of murine sarcoma virus, Harvey (MSV-H) and Moloney (MSV-M) strains, oncogenicity with the lactate dehydrogenase virus (LDV): Adult mice infected with LDV 3 days prior to MSV-H or MSV-M infection showed increased tumor incidence, reduction in the incidence of tumor regression, and increased incidence of death with tumor when compared to MSV-H or MSV-M infected controls. Tumor extract from mice inoculated with MSV-H alone was shown to be devoid of focus-forming activity on 3T3 cells when assayed in the presence or absence of "helper" virus. However, focus-forming activity could be demonstrated when an extract of tumors from mice infected with LDV and MSV-H was similarly assayed.
3. Activity of JLS-V5 STAS in vitro and in vivo: Attempts were made to verify and extend the findings of Jasmin et al. who isolated a new potent inhibitor of murine leukemia virus (JLS-V5 STAS) that showed antiviral activity both in vitro and in vivo. The inhibitor showed 99% reduction of virus titer when incubated with Moloney leukemia virus (MLV) in the XC plaque assay in vitro. JLS-V5 STAS showed potent activity in vitro when

added 2 hours before and up to 48 hours after MLV infection. A variety of "mock" preparations including saline and silicotungstic acid (STA) showed potent antiviral activity in vitro. Inhibitor prepared from JLS-V5 supernatant fluid, but substituting various protein precipitants for STA, showed little or no activity against MLV in vitro. These studies suggest that the common active ingredient in the various inhibitor preparations is STA. JLS-V5 STAS showed no activity against Friend virus infection or a Rauscher ascites tumor line in vivo. We have been unable to substantiate the existence of the proposed potent biological inhibitor of murine leukemia virus.

4. ALA synthetase activity in fibroblasts originating from porphyric patients and normals: ALA synthetase activity in fibroblasts originating from patients with acute intermittent porphyria (AIP) was shown to be increased compared to cultured fibroblasts from normal patients. Attempts to elucidate the genetic metabolic defect in porphyria was extended to studies of the electron transport system. A major difference was noted in the sensitivity of porphyric fibroblasts to rotenone, an inhibitor which reduces oxygen comsumption by blocking the transfer of reducing equivalents between NADH and cytochrome b.

5. Erythropoietin production in Rauscher virus (RV)-infected mice: RV infection is characterized by erythroblastosis, reticulocytosis, splenomegaly, and anemia. Despite the marked anemia, inappropriately low levels of erythropoietin were produced. Acute bleeding of already anemic RV-infected mice did not stimulate erythropoietin production. The data suggest that the chronic nature of the anemia may have lowered the threshold of response of erythropoietin production or that the virus infection damaged sites for erythropoietin production.

Significance to Biomedical Research and the Program of the Institute: The micro assay for ALA synthetase may be used as a possible diagnostic test for leukemia. Indirect assays suggest that the enzyme occurs in leukocytes from patients with acute myelogenous leukemia, acute monocytic leukemia, chronic myelogenous leukemia, but not in wbc's from patients with chronic lymphocytic leukemia nor in normal leukocytes. Investigations on heme synthesis in leukocytes may reveal the importance of porphyrin synthesis to the leukemic state.

Biochemical studies on fibroblasts from patients with the genetic disease acute intermittent porphyria could reveal enzymatic deficiencies in this disorder and could point to possible sites for therapy.

Proposed Course:

Further attempts will be made to maximize ALA synthetase activity in the IM-1 cell line in anticipation of testing leukocytes from patients with acute and chronic leukemia. New cell lines derived from normal and malignant white blood cells will be examined. Inducibility of the enzyme in IM-1 cells and cells derived from normal wbc's will be studied further. Studies will be conducted to determine the significance of the heme pathway in wbc lines. Further efforts will be made to elucidate the biochemical defects in porphyria with special emphasis upon possible aberations occurring in the electron transport chain.

Honors and Awards: None

Publications:

Ebert, P. S., Chirigos, M. A., and Chan, S. P.: Studies on the enzymology of the rhabdomyosarcoma induced by the murine sarcoma virus (Moloney). Cancer Res. 30: 960, 1970.

Ebert, P. S., Tschudy, D. P., Choudhry, J. N., and Chirigos, M. A.: A simple micro method for the direct determination of [^{14}C] δ-aminolevulinic acid production in murine spleen and liver homogenates. Biochim. Biophys. Acta 208: 236, 1970.

Turner, W., Ebert, P. S., Bassin, R., Spahn, G., and Chirigos, M. A.: Potentiation of Murine Sarcoma Virus (Harvey) (Moloney) Oncogenicity in Lactic Dehydrogenase-Elevating Virus-Infected Mice. Proc. Soc. Exp. Biol. and Med. (in press).

Serial No. NCI-4877
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Modification of normal and neoplastic cells by oncogenic and non oncogenic viruses.

Previous Serial Number: NCI-4832

Principal Investigator: Dr. Wilna A. Woods

Other Investigators:
Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. Willie Turner, VBB, NCI (NCI-4889)
Dr. John W. Pearson, VBB, NCI (NCI-4878)
Dr. Donald B. Schwartz, VBB, NCI (NCI-4879)
Dr. Gary Pearson, VBB, NCI (NCI-4880)
Dr. Harish C. Chopra, VBB, NCI (NCI-4874)
Dr. Peter Ebbesen, VLLB, NCI, Visiting Scientist

Cooperating Units: Inside NIH

Dr. Tom Cameron, CCNSC, NCI

Outside NIH

Dr. David Howell, Ft. Detrick, Frederick, Md.
Dr. Roger Johnson, Ft. Detrick, Frederick, Md.
Dr. C. A. Bowles, Hazleton Laboratories
Falls Church, Va.
Dr. Bruce Wright, Hazleton Laboratories
Falls Church, Va.
Dr. William Kerber, Hazleton Laboratories
Falls Church, Va.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(1) To investigate intracellular interactions between oncogenic and

non-oncogenic viruses *in vitro*.
(2) To investigate alteration of antigenic composition of tumor cell membranes by infection with non-oncogenic viruses.
(3) To investigate use of tumor cells with altered membrane antigenic profiles in immunotherapy and prophylactic vaccination.

Methods Employed:
A. *In vitro* systems:
1. A line of G+ lymphoblastic cells has been established *in vitro*, and has been immunologically and virologically characterized. This line is used for studies in the AKR spontaneous and transplantable tumor studies.
2. A line of tumor-inducing cells derived from a reticulum cell sarcoma of CDF_1 mice has been developed and characterized. A line of fibroblastic cells from the same tumor specimen has also been established. These cells will be used for colony inhibition and Cr^{51} release studies on cell mediated immunity in this tumor system, as well as in antigenic modification studies.
3. The transplantable canine sarcoma cell line 32043 from Hazleton Labs., Inc. has been used in dog tumor studies.
4. Separation of tumor cells from stromal cells in spontaneous and induced tumors by discontinuous Ficoll gradient separation is being applied to both mouse and dog tumor systems to more readily obtain tissue culture lines of spontaneous tumor cells for antigenic conversion studies.
5. ^3H-uridine incorporation into particles banding at 1.16 in a density gradient centrifugation is being used to identify presence of RNA in viral particles and demonstrate the presence of viral particles in cells.
6. Arsanilic acid and sulfanilic acid have been used to treat AKR-A cells in an attempt to chemically modify the cell surface.
7. Routine procedures used in this laboratory include virus titrations (focus formation by MSV-M, X-C, plaque formation by lytic virus, fluorescent focus for MuLV) and antigenic determinations (membrane fluorescence, cytotoxic antibody, fluorescent antibody staining of fixed cells, CF, hemagglutination inhibition, and neutralizing antibody titrations).
B. *In vivo* systems:
1. AKR and D_2AK mice are used for assay of AKR-A tumorigenicity and effectiveness of immunotherapy procedures.
2. CDF_1 mice are used for assay of the *in vitro* grown RCS tumor cell line and for immunotherapy experiments on this tumor.
3. BALB/c mice are used in MSV-M studies.
4. Beagle dogs are used for assay of immuno- and chemotherapy experiments at Hazleton Laboratories, Inc.

Major Findings
1. The infection of AKR-A cells with different non-oncogenic viruses has led to the establishment of carrier cultures with <1% of cells infected (NDV), lytic infection with production of infectious virus (Guaroa), lytic infection with production of antigen but no infectious virus (measles), infection and lysis of a small percentage of cells with spontaneous cure (MVM), carrier cultures with > 10% of cells infected and producing virus

(Germiston, Sindbis), or to no adsorption of input virus (Coxsackie). The myxovirus and arbovirus infections resulted in new viral antigens on the cell surface of a varying proportion of the cells. Measles and Germiston infection resulted in loss of G+ cellular antigen and gs-1 virion antigen in >90% of the cells; NDV resulted in loss of the GLV complex antigens in 50% of the cells. Only Germiston virus infected all cells in the carrier culture; the tumorigenicity of these cells has been lost. All other infected cultures were as tumorigenic as the parent culture.

2. The transplantable canine lymphosarcoma line 32043 was shown to support lytic infection with Edmonston measles virus. Measles antigen appeared on the cell membrane 4-5 days post infection; lysis of the culture with production of infectious virus occurred on day 7-10. Fibroblastic stromal cells from mixed cultures of ascites passages of these cells were not infected by the measles virus.

3. The reticulum cell sarcoma of CDF_1 mice carried in this laboratory has been established in tissue culture. From primary tumors, 2 cell types are seen in the cultures - a round loosely attached cell which grows in colonies and freely floating in the culture medium; and a fibroblast-like cell which grows in monolayer and readily forms colonies with a high efficiency. The round cells are tumorigenic in CDF_1 mice; the fibroblastic cells are not.

The parent in vivo line does not contain C-type particles by e.m. However, within 7 days of in vitro cultivation C-type particles can be found in both round and fibroblast-like cells. These cells give an X-C positive test, and contain gs-1 antigen by immunofluorescence, but do not infect Swiss mouse embyo fibroblasts as determined by both X-C assay and fluorescent antibody staining. 50% of the round cells have G+ cellular membrane fluorescent antigen. Supernatant from the cultures has not been oncogenic for CDF_1 sucklings in 3 months.

Lymph node cells from C57BL/6 mice immunized with the parent in vivo tumor and from CDF_1 mice bearing the tumor inhibit the formation of colonies of the round tissue culture cell line, but not of the fibroblastic cells. Both in vitro cell lines replicate vesicular stomatitis virus and are lysed by the infection. Immunization of CDF_1 mice with the virus and virus-infected cells is under way for immunotherapy studies.

4. Infectivity of C-type particles from the AKR-A cell line is being investigated. Within 6 months of initiation of the culture, AKR-A cells no longer released infectious GLV into the culture medium nor scored as infectious centers on cocultivation with Swiss mouse embryo fibroblasts. However, C-type particles are being produced both by budding from the plasma membrane and intracisternally in the cytoplasm. The cells are gs-1 positive and absorb neutralizing antibody from anti-GLV rat serum. ^3H uridine incorporation studies indicate presence of RNA in the particles. The particles adsorb to MEF, but are eluted immediately. They do not absorb to fresh AKRA cells nor to dog embryo fibroblasts. This suggests that the block may be one of penetration. Further studies are underway.

5. In cooperation with Dr. Peter Ebbesen, protection studies in AKR mice were undertaken using his Copenhagen virus, which appears to be a Gross type MuLV by neutralization. Mice were vaccinated with increasing doses of Copenhagen virus. Part of the mice were challenged with AKR-A cells

1 month after vaccination was complete; no protection was produced. The remaining mice are being held for incidence of spontaneous leukemia.

6. In cooperation with Dr. Donald Schwartz, a continuous line of cells was derived from an MSV-M induced tumor of BALB/c mice. The culture is tumorigenic in BALB/c mice; it is producing defective MSV-M virus which produces lethal tumors in suckling BALB/c mice. Adult BALB/c mice are resistant to tumor induction by the virus; tumor was induced in only 1/5 adult mice, at 90 days after inoculation. Further studies on the vaccinating potential of the virus and its defectiveness are underway. The line is also being used for studies on cellular immunity in the BALB/c-MSV-M system using Cr^{51} release as cytotoxic assay.

7. Miss Karen Whedon, a COSTEP medical student, investigated the chemical alteration of AKR-A cell membranes. Both sulfanilic and arsanilic acid modified the cell membrane and eliminated tumorigenicity of the cells. No protection against tumor induction was elicited in DBA mice; in fact a slight enhancement was noted. The system will be investigated further as personnel are available.

8. In cooperation with Dr. John Pearson, AKR-A cells were lethally irradiated and tested for immunogenicity in AKR mice. Results indicate that irradiation alone does not confer vaccinating potential on these cells in the homologous system.

9. In cooperation with Dr. David Howell and Dr. Roger Johnson at Ft. Detrick, studies are underway to determine the possibility of using various arboviruses for antigenic conversion of AKR-A cells. Results to date indicate that the cells are more sensitive to the arbovirus group than to the myxovirus group. Guaroa virus lyses all cells of an infected culture. Germiston and Sindbis enter a latent phase, after which 10-100% of the cells score as infectious centers by plaque assay. AKR mice are not susceptible to the Germiston virus when injected with AKR-A-Germiston-infected cells, and such mice are protected from challenge with $10LD_{50}$ of virulent Germiston virus. The Germiston-infected AKR-A cells are not tumorigenic in AKR mice and have lost G+ antigens. Protection against non-infected tumor cells with infected AKR-A cells is now being investigated.

Significance to Biomedical Research and the Program of the Institute: The AKR spontaneous leukemia system is a useful model for the study of human lymphoid leukemogenesis. Immunotherapy studies on the AKR virus-induced lymphomas have direct application in the formulation of similar systems for human immunotherapy. Further, an understanding of the mechanism of viral immune oncolysis may be useful in the control of human leukemias. The interaction of oncogenic and non-oncogenic viruses must be determined to insure complete safety in such cases. In this respect virus-virus interactions may also play a part in leukemogenesis for any animal species including man. The investigation of non-infectious C-type particles for use in vaccination programs using live virus will be directly applicable to human vaccination studies. Use of such viruses for interference with ongoing infections may also prove to be useful therapeutically.

Proposed Course:

Therapy studies will be directed toward the elucidation of the underlying mechanisms of immune reactions. Chemical, physical and biological alteration of tumor antigens in murine, canine and bovine tumor systems will be explored.

Studies on the possibility of viral "transduction" of oncogenes will be actively pursued. In addition, interference studies with non-infectious virus will be pursued.

Honors and Awards:

None

Publications:

Woods, W. A., Wivel, N. A., Massicot, J. G. and Chirigos, M. A.: Characterization of a rapidly growing AKR lymphoblastic cell line maintaining gross antigens and viral replication. Cancer Res. 30:2147-2155, 1970.

Woods, W. A., Massicot, J., and Chirigos, M. A.: An immunofluorescent focus assay for Gross leukemia virus. Proc. Soc. Exp. Biol. and Med. 135: 772-777, 1970.

Serial No. NCI-4878
1. Viral Biology Branch
OASDVO, Etiology
2. Virus & Disease Modification
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project report
July 1, 1970 through June 30, 1971

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical and Physical Methods.

Previous Serial Number: NCI - 4834

Principal Investigator: Dr. John W. Pearson

Other Investigators:
Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. Willie Turner, VBB, NCI (NCI-4889)
Dr. Paul Ebert, VBB, NCI (NCI-4876)
Dr. Wilna Woods, VBB, NCI (NCI-4877)
Dr. Donald Schwartz, VBB, NCI (NCI-4879)

Cooperating Units:

Inside NIH

Dr. G. Canellos, Medicine Branch, NCI
Dr. R. Adamson, LCP, NCI

Outside NIH

Dr. G. Spahn, Microbiological Associates, Inc., Bethesda, Maryland
Dr. John Landon, Bionetics Research Laboratories, Kensington, Maryland
Dr. Charles Rickard, Cornell University, Ithaca, N. Y.

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

The overall aim of this project is to effectively control virus induced, spontaneous and transplantable leukemias and lymphomas by several therapeutic approaches. The therapeutic approaches encompass individual or combination drug therapy, immunotherapeutic methods, vaccines, and the application of interferon. Any therapy leading to effective control will be submitted for pre-clinical testing, evaluation and eventual clinical trial.

Methods Employed:

Virus and Tumor Animal Systems:

1. The Friend, Rauscher, Moloney leukemogenic viruses, the Moloney sarcoma virus, and the plasma passage virus variant (MSV-PV) are employed for *in vivo* studies.
2. A Moloney leukemia virus transplantable solid tumor and ascites line, a Graffi leukemia virus transplantable tumor line, a sarcoma transplantable tumor line of MSV(PV) and a transplantable tumor line induced by Gross virus are employed for *in vivo* studies in mice. A Dunning lymphoma transplantable tumor maintained in Fisher rats is being utilized for *in vivo* studies.
3. AKR mice, which develop spontaneous lymphoma.

Major Findings:

1. Chemo-Immunotherapy: Investigations are currently underway to achieve, prolong, and hopefully complete remission in diseased animals following the application of chemo-immunotherapy.

a. Moloney ascites tumor line (LSTRA). Studies have shown that s.c. inoculation of 1 X 10^4 LSTRA cells into CDF_1 mice induces 100% tumor with death occurring 12-14 days post inoculation. Based on periodic bioassays of spleen and blood the disease is first detected systemically on day 7 following s.c. inoculation of 1 X 10^4 LSTRA cells. Tumors (5-7 mm) also appear at the site of inoculation at this time. Administration of 25 or 30 mg/kg of BCNU on day 7 results in a period of remission of 12 days before relapse occurs. Preliminary studies have shown that administration of immune spleen cells to drug treated animals when in a state of remission resulted in 94% survivors at the time the experiment was terminated (70 days) as opposed to 40% survivors in the drug treated group. Similarly, drug treated animals exposed to BCG or an inactivated Moloney virus vaccine exhibited survivors of 80 and 87%, respectively. Studies are now underway to determine the best time during remission to administer non-specific enhancers or vaccines alone or in combination. Likewise, treatment with single or mutiple injections of immune cells at different periods during remission is being investigated.

b. Dunning transplantable lymphoma line. Studies are currently in progress involving a highly fatal transplantable leukemia in rats. Subcutaneous inoculation of 2.0 X 10^4 cells resulted in a palpable tumor by day 10 followed by death between the 20th and 24th day. When the animals were near death the white count was found to be elevated sometimes as high as 400,000 cells per mm^3. Cytoxan therapy has been shown to be very effective against this disease. Tumor bearing rats (12-17 mm) treated with 50 mg/kg of cytoxan resulted in complete regression of the tumor at the site of inoculation 6 days following therapy. The period of remission following drug therapy has been observed to be approximately 12 days before the reappearance of the tumor at the original site of inoculation. Studies are now underway to determine the best time during the remission period to administer non-specific enhancers or vaccines alone or in combination. Likewise, treatment with immune spleen cells and interferon stimulators at different periods during remission is being investigated.

e. Similiar chemo-immunotherapeutic studies as described above are being carried out with the Graffi leukemia virus transplantable tumor line and a transplantable tumor line induced by Gross virus.

2. Antiviral Therapy:

a. A reported viral inhibitor recovered from silicotungstic acid-treated supernatant fluid of JLS-V5 cells grown in tissue culture and referred to as JLS-V5(STAS) was investigated for activity *in vivo* against FLV induced disease as well as for Rauscher lymphoid leukemic ascites cells. The parameters utilized to test the effectiveness of the JLS-V5(STAS) preparations administered prior to or after infection were retardation of splenomegaly, inhibition of tumor growth and an increase in median survival time. JLS-V5(STAS)failed to have an inhibitory effect against Friend leukemia. On the contrary, an enhanced splenomegalic response as well as shorter median survivor times were observed in the FLV-infected mice when compared to the splenomegalic response attained in FLV controls. Similarly, preincubation of Rauscher leukemia ascites cells *in vitro* with JLS-V5(STAS) failed to alter the tumor-inducing capability of the ascites tumor in mice.

b. Approximately 20 compounds submitted to us from different sources were screened for antiviral activity against Friend leukemia virus. None of the compounds were found to be significantly active against FLV. The parameters utilized to test the effectiveness of the compounds were retardation of splenomegaly, inhibition of virus replication based on plasma bioassays, and an increase in median survival time when compared to the untreated control group.

3. Interferon: The double-stranded complex of polyinosinic and polycytidylic acids (pI:C) used alone and in combination with poly-D-lysine has been employed in interferon studies *in vivo*. Mice infected with the highly tumorigenic murine sarcoma plasma virus (MSV-PV) followed by treatment with either pI:C or pI:C/poly-D-lysine resulted in long term survivors when compared to the untreated MSV(PV)-infected group. Interestingly, infected mice treated with pI:C/poly-D-lysine exhibited survivors of 73% as compared to 46% survival in the pI:C treated groups when the study was terminated at 150 days. The median survival time of the infected MSV(PV) control was found to be 56.0 days with one animal surviving at the termination of the study. Studies are currently in progress where different regimens of interferon treatment are being applied following MSV(PV) infection. Similarly, pI:C and pI:C/p-D-lysine are being administered to animals when in a state of remission following drug therapy.

4. Immunology: A formalin-treated extract of spleens of mice infected with MSV(PV) virus was used to prepare a vaccine. Animals were immunized once a week for 3 weeks followed by challenge with varying dilutions of MSV(PV) ten days after the last immunizing injection. The noninfectious vaccine was shown to provide effective resistance against MSV(PV) regardless of the challenge dose. Similarly, MSV(PV) immunized mice were observed to exhibit protection against challenge doses of FLV and MSV(M).

In Vitro neutralization studies are now in progress utilizing MSV(PV) antiserum against MSV(M), MSV(H), MSV(K) as well as the homologous virus in newborne mice.

Significance to Biomedical Research and the Program of the Institute:
These studies are directed toward the prevention, treatment and control of virus-induced or spontaneous leukemias, lymphomas, and sarcomas in animal systems. The use of drugs, either alone or in combination, synthetic interferon inducers, immunotherapy and vaccines may lead to effective therapy applicable to the control of human neoplasia.

Proposed Course:
Research in each of the areas described under objectives will continue. More emphasis will be placed in maintaining prolonged periods of remission following chemotherapy. The use of non-specific enhancers, vaccines, immune cell transfer, interferon stimulators and other immunological factors will be investigated in both mouse and rat leukemia and sarcomas systems following drug therapy. Similarly, long term studies to determine the effect of non-specific enhancers as well as interferon stimulators on AKR mice of different ages are being planned. It is hoped that information gained from these investigations may be applied toward the control of human cancer.

Honors and Awards:
None

Publications:
Pearson, J. W., Gibson, W. T. and Chirigos, M. A. Use of a murine sarcoma virus in an *in vivo* assay for antiviral and antitumor agents. *Cancer Res.* 30:2024-2028, 1970.

Serial No. NCI-4879
1. Viral Biology Branch
OASDO, Etiology
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Attempts to Enhance Cellular Immunity with BCG

Previous Serial Number: None

Principal Investigator: Dr. Donald B. Schwartz

Other Investigators: None

Cooperating Units:
Dr. Michael A. Chirigos (NCI-4875)
Dr. Wilna Woods (NCI-4877)
Dr. B. Zbar, BGY, NCI

Man Years:

Total:	1.0
Professional	1.0
Other:	0.0

Project Description

Objectives:

1. To develop, using a primary virus-induced murine sarcoma, a system demonstrating tumor inhibition with BCG *in vivo*.
2. To determine the mechanisms of BCG's activity by *in vivo* manipulations.
3. To study the system *in vitro* using newly established techniques.
4. To apply the techniques to other virus-induced tumors.

Methods Employed:

A. *In vitro* system: Primary tumors are induced in mice with a pool of murine sarcoma virus of Moloney (MSV-M). BCG (a strain of attenuated Mycobacterium bovis) is injected into the mice at various times, and the effect of BCG on tumor growth is observed.

B. *In vivo* system: A line of MSV-M tumor cells is maintained in tissue culture. Assays of cellular immunity, cytotoxic antibody, and neutralizing antibody will be done with sera and lymphocytes from the mice described in A.

Major Findings:

A. *In vitro* system: Mice injected with BCG 28 days before infection with

MSV-M had shorter latent periods for tumor development and longer survivals than mice not previously exposed to BCG. A mixture of BCG with the virus inoculum decreased the incidence of tumors in inoculated mice, while in mice that had previously been inoculated with BCG, the mixed inoculum did not induce tumor development. The BCG treated mice that did not develop tumors when injected with the virus were resistant to a challenge with a lethal dose of MSV-M.

B. In vivo system: Techniques to measure cellular immunity and cytotoxic antibody are presently being developed using measurements of release of radioactive Cr-51 from labelled tumor cells. It has been established that tumor cells do take up and retain the radio-label. Tests for antibody and cellular immunity are pending.

Significance to Biomedical Research and the Program of the Institute:

Recently there has been considerable interest in the use of BCG therapy of malignancies. A better understanding of BCG in its therapeutic milieu is necessary, requiring animal systems to study the activity of BCG. Such a system has been developed using primary virus induced tumors which demonstrates effective protection of mice against the development of virus-induced neoplasms. The possibility of immunizing other species of animal against oncogenic virus using BCG manipulations may be possible; the ultimate goal of human immunizations seems realistic. In the meantime, a better understanding of the mechanisms of BCG's activity can be obtained by studying this mouse system in detail.

Proposed Course:

Studies on the mouse system described will be continued and these studies will be extended to other species of animal.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4880
1. Viral Biology Branch
OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunological and Virological Studies on Human and Animal Tumors

Previous Serial Number: None.

Principal Investigator: Dr. Gary Pearson

Other Investigators: Dr. Jack Pearson, VBB, NCI (NCI-4878)
Dr. Wilna Woods, VBB, NCI (NCI-4877)

Cooperating Units: Dr. John Landon, Bionetics Research Laboratories, Kensington, Maryland
Dr. William Kirber, Hazelton Laboratories, Falls Church, Virginia

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description

Objectives:

(A) To establish an animal model in primates for study of EB virus infections and to develop *in vitro* methods to investigate cell-mediated immunity against EB virus associated antigens.

(B) To attempt to transfer cellular immunity against viral and/or tumor antigens with soluble factors from immune lymphoid cells.

Methods:

(A) Infection of primates with EB virus:
Buffy coat cells from Rhesus and Cynomologus monkeys found negative for antibodies to EB virus have been co-cultivated with EB virus containing human cells previously X-irradiated (4500 r). Cultures will be followed

for evidence of infection and transformation. If positive, these cells will be reinoculated into donor animals.

(B) Transfer of cellular immunity:
C-57 Bl mice were immunized with mouse sarcoma virus (MSV-M). Half were exposed to 800 r X-irradiation. Three days later plasma from both irradiated and non-irradiated immune groups were injected into two groups of normal mice. These animals together with a control group were challenged with a Moloney virus-induced tumor three days later.

Soluble factors of dogs hyperimmunized against a canine tumor were injected into dogs less than five days old. Three days later the animals were challenged with sufficient tumor cells to elicit a 100 percent tumor response in animals less than ten days old. Experimental animals will be observed for tumor development.

Major Findings:

This is a new project. Insufficient time has elapsed to evaluate results.

Significance to Bio-Medical Research and the Program of the Institute:

It is important to establish EB virus infection in an experimental animal system to determine whether any evidence of a direct causal relationship exists between EB virus infection and oncogenesis in man.

The role of immune lymphocytes in the rejection of tumor grafts is well established. To eliminate problems associated with whole cell transfer in outbred populations it is important to evaluate specific transfer of immunity with soluble factors.

Proposed Course:

Present studies will continue. Further attempts will be made to demonstrate cellular immunity *in vitro* against EB virus antigens in human and primate systems. If a delayed hypersensitivity reaction to EB virus antigens can be induced in primates, attempts will be made to transfer reactivity by soluble factors to normal animals. Different species of primates will be inoculated with infectious EB virus, concentrating on the route of inoculation, immunosuppression and co-factors such as other viruses.

Other Activities:

Project Officer on one research contract and Assistant Project Officer on five research contracts within the Special Virus Cancer Program.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4881
1. Viral Biology Branch
   OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies with Epstein-Barr Virus (EBV). Host-Virus-Cell Relationships.

Previous Serial Number: NCI-4893

Principal Investigator: Dr. Virginia C. Dunkel

Other Investigators: Dr. Sam Margolis (NCI-4882)

Cooperating Units: None.

Man Years:

Total:	5.0
Professional:	1.0
Other:	4.0

Project Description

Objectives:

1. To study the nature of soluble antigens derived from EBV-infected and EBV-free cell lines and primary Burkitt lymphoma tissue.

2. To determine the antibody status of primates and whether sero-negative animals represent a suitable experimental animal for EBV studies.

Methods Employed:

The following methods are being applied to these studies: Immunofluorescence, complement-fixation, micro immunodiffusion, immunoelectrophoresis, disc electrophoresis, column chromatography, and infectivity tests. Soluble antigens are being prepared from large batches of tissue cultures and primary tumor specimens. Sero-negative animals have been inoculated with EBV.

Major Findings:

Sera from wild caught rhesus, cynomolgus, bonnet, African green and Talapoin monkeys contained antibodies which cross-reacted with EBV in the HR1-K and EB-3 cell lines as determined by immunofluorescence (IF). The same sera were also reactive in immunodiffusion (ID) with soluble EBV-associated antigens. Further immunodiffusion tests with selected sera revealed that they all reacted with the Beta antigen found in such cell extracts and gave a line of identity with human sera. In contrast to these results obtained with Old World monkeys, sera from New World monkeys (Owl, Squirrel and golden marmoset) were all negative by IF and ID for antibodies to EBV. In order to further clarify the antibody status of these monkeys studies are underway to determine whether these IF and ID positive sera can neutralize the virus.

An organized and controlled study of the responsiveness of Macaque monkeys to EBV was completed. Thirty animals were used in this study. Prior to inoculation it was determined that they were negative for antibodies to EBV. Experimental and control animals were inoculated by the intravenous or intranasal route. Sera from these animals were tested for the presence of EBV-related antibodies by IF, ID, and for the presence of heterophile antibody by agglutination of sheep erythrocytes. By the fourth week post-inoculation, sera from all animals inoculated intravenously with EBV showed positive immunofluorescent staining against EBV-infected cells. In addition three of five animals inoculated intravenously with a media control preparation showed a similar staining pattern. Those animals inoculated intranasally were less responsive. One of nine of the test animals and one of five of the control animals responded. Only sera from those animals inoculated IV with EBV were positive in the immunodiffusion test. Four of nine animals were positive two weeks post-inoculation and this increased to six of nine by ten weeks post-inoculation. Sera from all animals inoculated intravenously agglutinated sheep erythrocytes. All activity was removed by adsorption with guinea pig antigen, thus indicating that it was not a heterophile response.

In order to determine whether the EBV genome might have been incorporated into any lymphoid cells and could be detected by tissue culture methods, eight test animals (EBV-IV) and four control animals were sacrificed. Peripheral leukocytes, splenic leukocytes and kidney cells were placed in culture. No cultures were established from the peripheral or splenic leukocytes. All cultures grown from kidney cells were negative by IF for EBV-antigen(s).

Extracts from nine Burkitt tumors were tested for antigenic activity by immunodiffusion and complement fixation. The extract from one tumor was positive in immunodiffusion with known positive sera. A variety of tissues obtained from autopsies of Burkitt lymphoma patients were also extracted and checked for antigenic activity. In one case, heart, thyroid, kidney, liver, mesenteric lymph node, and lung showed no reactivity in immunodiffusion tests while spleen and tumor extracts from the same patient were positive. In a second case tonsil, bone marrow, liver, and lung extracts were all negative. The majority of the extracts were anti-complementary.

Continuing studies with cell-associated antigens are indicative of the complex nature of the EBV-lymphoblastoid system.

Significance to Biomedical Research and the Program of the Institute:

The availability of an experimental primate sensitive to infection by EB virus would be extremely helpful in determining the oncogenic properties of this agent. The work done shows that Old World monkeys are susceptible to an agent very similar to the EB virus infecting man. The difficulty in isolating the virus of Macaques and their apparent insensitivity to infection with EB virus suggests that another species may be of greater value to provide a working animal model system for EB virus.

Studies on the localization of EB virus in human organs support the specific predilection of this virus for lymphoid tissue.

Proposed Course:

Other primate species will be studied in an effort to obtain an animal responsive to EB virus infection. Further analyses will be undertaken to determine whether antibody reactions in EB virus inoculated animals give rise to antibodies directed against any of the complex EB virus antigens. Investigations will continue on the nature of cell-associated EBV antigens.

Other Activities:

Project Officer on four and Assistant Project Officer on two research contracts within the Special Virus Cancer Program.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4882
1. Viral Biology Branch
OASDVO, Etiology
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigations on the Biochemical Composition and Growth Kinetics of Mammalian Tumor Viruses.

Previous Serial Number: None.

Principal Investigator: Dr. Sam Margolis

Other Investigators: Dr. Virginia C. Dunkel (NCI-4881)

Cooperating Units: None.

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To study the composition of the Mason-Pfizer Monkey Virus (M-PMV) particle, the kinetics of the synthesis of its subunits, and the relationship of the synthesis of macromolecules to the morphogenesis of the particle.

2. To determine the relationship of the above parameters to those of othe oncogenic viruses.

Methods Employed:

The following methods are being applied to these studies: Density gradient fractionation, polyacrylamide gel electrophoresis, RNA and protein fractionation, hemagglutination, indirect immunofluorescence, and radiolabeling.

Major Findings:

These studies are in the preliminary stages of development. However, the following has been observed:

The M-PMV has been isolated from sucrose density gradient purified virus (density 1.66 gm/cc). The RNA, when analyzed by polyacrylamide gel electrophoresis appeared to have the same molecular size as avian myloblastosis virus and Moloney leukemia virus. Calculation of the molecular size of M-PMV RNA based upon molecular size of 18 S, 28 S, and 45 S cell RNA standards indicated that the molecular weight was 7.4×10^6 daltons. Using the Spirin formula, $MW = 1550 (S_{20w})^{2.1}$, an estimated sedimentation value of 56 S was calculated. This does not agree with reported S_{20w} values of 65-70 S which were calculated from sedimentation velocity data. Sindbis virus RNA shows similar inconsistencies. The basis of these variations are currently under investigation.

Unpublished data from Pfizer, Inc. indicated the M-PMV can be assayed in both monkey embryo or NC37 cells. In our hands, monkey embryo cells chronically infected react with rabbit anti M-PMV absorbed with normal monkey embryo cells. Using the indirect immunofluorescence test a distinct perinuclear fluorescence was observed. In NC37 cells chronically infected with M-PMV, no fluorescence was observed when rabbit anti M-PMV serum (adsorbed with NC37 cells) was used.

Monkey embryo cells, chronically infected with M-PMV have also been evaluated for hemadsorption of human O, guinea pig, and gander red cells. No hemadsorption was observed. This suggests that the widely distributed SV_5 virus is absent from our cultures. In addition, preliminary serological studies with foamy virus pseudotypes 1, 2, 3, 5, 6, and 7 and anti M-PMV serum indicate the absence of antigenic cross-reactivity between the M-PMV and the monkey foamy viruses. Studies with antisera to foamy viruses and M-PMV chronically infected monkey embryo cells are currently in progress. Together these data minimize the possibility that the M-PMV material may contain the common RNA virus contaminants.

Significance to Biomedical Research and the Program of the Institute:

The M-PMV is the first C-type particle to be isolated from a primate mammary tumor and propagated in tissue culture. The morphogenesis of this virus is different from that of the mouse mammary tumor virus (MTV) in that the final virus particle of M-PMV is structurally similar to the mature C-type particle and not the B-type particle characteristic of MTV. Both viruses have an early A-type particle stage and both have approximately the same density. Morphologically the final C-type particle resembles those of RNA tumor viruses and it may thus be the first oncogenic "C-type" virus of primate origin. Biochemical analysis of this virus will permit the elucidation of the kinetics of the synthesis of the viral components and correlation of such information with the morphogenesis of the particle. It will also allow in-depth comparison of M-PMV with known oncogenic viruses.

Proposed Course:

Further isolation and characterization of the nucleic acid and proteins of M-PMV will be carried out to acquire more information on the biochemical nature of this virus and its relationship to other RNA tumor viruses.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4883
1. Viral Biology Branch,
OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970-June 30, 1971

Project Title: Immunity to Virus-Induced Tumors

Previous Serial Number: NCI-4905

Principal Investigator: Dr. Charles W. Boone

Other Investigators: Dr. Meera Paranjpe

Cooperating Units: None

Man Years:

Total	4.2
Professional:	1.4
Other:	2.8

Project Description

Objectives:

1. To develop *in vitro* assays for measuring cell-mediated immunity to tumors.

2. To determine the cellular mechanisms by which anti-tumor immunity is stimulated and expressed.

3. To isolate and biochemically characterize the virus-induced surface antigen(s) of neoplastic cells.

Methods Employed:

1. Mass production of monolayer and suspension-cultured cells.

2. Enhancement of immunogenicity of tumor homogenates by prior infection of the cultured tumor cells with influenza virus.

3. *In vitro* culture of target tumor cells and anti-tumor immune lymphocytes.

4. Paired radiolabel antibody adsorption method for quantitative assay of cell surface antigens.

5. Application of isotopic methods to determine tumor cell-induced lymphocyte stimulation and lymphocyte-mediated target tumor cell destruction.

Major Findings:

1. Specific immunization against tumor challenge with homogenates of tumor cells previously infected with influenza virus. SV40 transformed tumor cells were infected in culture with influenza-virus, then homogenized by freeze-thaw and sonication. Injection of this cell free tumor cell homogenate into mice rendered them solidly immune to challenge with viable tumor cells. Inoculation of non-influenza virus-infected tumor cell homogenate, or of influenza virus-infected normal cells, was without effect.

2. Specific stimulation of immune lymphocytes in vitro by syngeneic tumor cells. Balb/c mice were made immune to challenge with syngeneic SV40-transformed tumor cells. When their spleen lymphocytes were incubated *in vitro* with the target tumor cells, lymphocyte transformation occurred as determined by tritiated thymidine uptake. Incubation of the immune spleen lymphocytes with non-SV40-transformed tumor cells, or with normal cells, did not induce lymphocyte transformation.

3. Target tumor cell destruction in vitro by syngeneic lymphocytes. Cultures of SV40-transformed tumor cells were prepared and labeled with tritiated thymidine. When they were thereafter incubated with immune spleen cells, destruction of the monolayers by immune spleen cells, but not by normal spleen cells, occurred.

4. The cell surface antigen detected in EB virus-infected lymphoid cells by membrane immunofluorescence is not integrated into the membrane structure by covalent bonds. It was shown that during separation of plasma

membranes from cells containing the EB virus antigen, the antigen is lost from the membrane surfaces by simple dilution. It appears that the so-called surface antigen may represent virus components which are physically adsorbed to the cell surface membrane on either its external or internal aspect.

5. By means of the paired radioiodine-labeled antibody adsorption assay, the number of antigenic sites on HeLa cell surfaces was counted, the concentration of antibody in immune serum was measured, and the association constant of the antibody-cell surface antigen was determined.

6. Study of surface antigens of AKR rat lymphoma cells demonstrated that it is possible to measure the number of antigenic sites per cell and the ΔF of antibody binding. The feasibility of using the paired radiolabel antibody assay to screen human leukemic cells for cross-reacting antigens with AKR virus-infected cells was demonstrated.

Significance to Biomedical Research and the Program of the Institute:

1. The finding that the immunogenicity of a tumor cell homogenate can be enhanced by prior infection with influenza virus has considerable potential for the immunotherapy of human tumors. Primary tumor tissue which is removed from the patient, infected with influenza virus, homogenized, inactivated with regard to infectivity, and then inoculated back into the patient, could conceivably enhance the patient's immune resistance to residual tumor and to the development of metastases.

2. The development of effective *in vitro* assays of cell-mediated immunity against tumors is urgently needed for monitoring the immune response of human patients to their own tumors during the course of conventional therapy (surgery, x-irradiation, chemotherapy). If immunotherapy in humans is one day contemplated, these *in vitro* assays will form the basis for detecting changes in the patient's immune responses.

Proposed Course: The work involving EB virus membrane antigens will be terminated in the ensuing year. Other than this, the work will continue as described above without major changes.

Honors and Awards: None

Publications:

Boone, C.W., Blackman, K., and Brandchaft, P.: Tumor immunity induced in mice with homogenates of influenza-virus-infected tumor cells. Nature (In press).

Boone, C.W., Irving, D.N., and Rubinstein, S.: Quantitative studies on the binding of antibody to the surface of HeLa cells. J. of Immunol. (In press).

Serial No. NCI-4884
1. Viral Biology Branch,
   OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970-June 30, 1971

Project Title: Spontaneous and Virus-Induced Neoplastic Transformation *In Vitro*

Previous Serial Number: NCI-4900

Principal Investigator: Dr. Mitchell H. Gail

Other Investigators: Dr. Charles W. Boone

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.2
Other:	0.8

Project Description

Objectives:

To quantitatively define the specific functional and structural attributes of cells undergoing spontaneous and virus-induced neoplastic transformation.

Methods Employed:

1. Monolayer and suspension cell culture.

2. Tumorigenicity assays of various neoplastic cell lines in syngeneic mice.

3. Time lapse cinephotomicrography of cultured cells.

Major Findings:

1. Cultured normal and SV40 virus-transformed fibroblasts execute a random walk.

2. A method has been found to quantitatively measure contact inhibition of locomotion. The mean square displacement of fibroblasts on the surface of the culture vessel is directly proportional to the elapsed time and inversely proportional to the area cell density. A plot of mean square displacement versus time produces a straight line with a given slope, D. The constant, D, is inversely proportional to cell area density. If D versus cell area density is plotted, another straight line is obtained with a negative slope. This slope precisely and quantitatively measures contact inhibition of locomotion.

3. Contact inhibition of locomotion is NOT generally correlated with the degree of malignancy of a tumor, as measured by tumorigenic dose. A number of cultured cell lines which readily produced tumors exhibited marked contact inhibition of locomotion, whereas others which were practically nontumorigenic showed little contact inhibition of locomotion.

Significance to Biomedical Research and the Program of the Institute:

Research on virus-induced neoplasia in recent years has focused primarily on the oncogenic virus-cell interaction with the expectation of unearthing leads to the virus causation of human cancer. The intent of this project is to focus on phenotypic changes in the virus-transformed cell itself which determine neoplastic cell behavior *in vivo*, with the expectation of eventually unearthing leads to cancer therapy.

Proposed Course: To continue as described above with no significant changes.

Honors and Awards: None

Publications:

1. Gail, M. H. and Boone, C. W.: The locomotion of mouse fibroblasts in tissue culture. Biophys. J. 10: 980-993, 1970.
2. Gail, M. H. and Boone, C. W.: Density inhibition of motility in 3T3 fibroblasts and their SV40 transformants. Exptl. Cell Research 64: 156-162, 1971.

Serial No. NCI-4885
1. Viral Biology Branch
   OASDVO, Etiology
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Viruses in Human and Animal Tumors

Previous Serial Number: NCI-4884

Principal Investigator: Dr. Sarah Stewart

Other Investigators: Mrs. Elizabeth Mitchell, Electron Microscopy Section

Cooperating Units: Electron Microscopy Section, Viral Biology Branch, NCI

Man Years:

Total:	1.0
Professional:	0.2
Other:	0.8

Project Description

Objectives:

(A) To study human neoplasms and attempt recovery of viruses of possible etiologic significance.

(B) To determine whether a virus is etiologically associated with renal carcinomas in Claude BALB/c strain mice.

Methods Employed:

(A) Biopsy specimens of sarcomas, Wilms' tumor, Hodgkins' disease, glioblastoma, and mammary tumors as well as leukemia buffy coat cells were cultured. Portions of the biopsy specimens were examined for virus by electron microsocpy culture fluids and cells were examined periodically for virus particulates by electron microscopy and by immunofluorescence for reactivity with sera from the tumor donor and patients with similar tumors. Cell-free supernatant fluids from cell lines that had undergone spontaneous transformation were tested for focus formation on monolayered cultures of W138 cells and primary human amnion cells. The culture fluids were cleared of all cells by centrifugation only and by centrifugation and filtration through 450 mm filters.

(B) Virus of the A and B types had been previously detected in an established cell line of renal carcinoma from Claude BALB/c mice. Cell-free filtrates from this culture were tested for tumor induction in BALB/c mice of the Heston subline, in BALB/c mice of the Claude subline and in crosses of these two lines. The fluids were also tested for cell transformation of primary embryonic kidney cultures made from the Claude subline.

Immunofluorescence and EM studies were carried out periodically on the established renal carcinoma cell line.

A pure bred high renal tumor line was established from the Claude mice for virus studies.

Major Findings:

(A) Four human tumor cell-lines cultured for periods ranging from two months to two years transformed spontaneously. The cultures had all been growing as monolayered fibroblasts until the transformation occurred. They were derived from the following:

(1) A glioblastoma, two years in culture. (2) A primary osteosarcoma, 13 months in culture. (3) A lung metastasis from an osteosarcoma, 12 months in culture. (4) A rhabdo myosarcoma, two months in culture. The biopsy specimen from this tumor was found to have a virus particle which budded from the cell membrane. Virus has not been observed in the cultures nor in any of the other cultures examined.

Experiments for demonstrating cell transformation of W138 and human amnion cells have all yielded positive results with fluids that were centrifuged only, but persumably were cell free. With filtered fluids, transformation was demonstrated only occassionally and then only after 60-90 days. With the unfiltered fluids the transformation occurred after 21 days.

Virus Rescue experiments are at present being conducted with the above cell lines.

(B) The Claude renal carcinoma cell line which is being examined by electron microscopy continues to shed virus of the A and B types. This virus appears to cross with the mammary tumor virus as shown by immunofluorescence.

Mouse embryonic kidney cultures were found to transform when treated with cell-free filtrates of the established renal carcinoma culture. It has not been determined if these are epithelial or fibroblast cells or if they have virus.

The final results on the inoculated mice have not been analyzed.

Significance to Biomedical Research and the Program of the Institute:

(A) The viral induction of leukemia and sarcoma in a number of different animal species is well known. In man, less direct methods must be applied. Although efforts to date have not resulted in the isolation of agents known to be oncogenic in man, it is highly unlikely that human tumors are unique in this respect.

(B) The high incidence of renal carcinoma in the Claude subline of BALB/c mice suggest the possibility that a virus is etiologically involved. The results obtained with a virus preparation from cultured renal tumor strongly suggests carcinogenic and leukemogenic activity.

Proposed Course:

The principal investigator has left the Public Health Service.

Honors and Awards:

None.

Publications:

None.

Serial No. NCI-4886
1. Viral Biology Branch,
OASDVO, Etiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970-June 30, 1971

Project Title: The Valency of Antibody Binding to the Surface of HeLa Cells

Previous Serial Number: None

Principal Investigator: Mr. Robert J. Koegel

Other Investigators: Dr. Charles W. Boone

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	1.2
Other:	0.5

Project Description

Objectives:

To determine whether antibodies bind to the surface of HeLa cells in the monovalent or bivalent configuration, i.e., with one combining site or both combining sites.

Methods Employed:

Antibodies against the HeLa cell surface are chemically modified to make hybrid antibodies, in which only one of the two combining sites is specific for the HeLa cell. The binding affinity of these antibodies, i.e., the ΔF^{o} of binding, is measured by techniques developed in this laboratory, and compared with the binding affinity of natural antibody which has two combining sites.

Serial No. NCI-4886

Major Findings:

During the last three months since the inception of this project, Fab_2 fragments were prepared. Work has proceeded on the reduction of the Fab_2 dimer and recombination of the monomeric units to make monovalent hybrid dimers.

Significance to Biomedical Research and the Program of the Institute:

This project will make available knowledge and techniques for studying the problem of blocking factors in serum which prevent tumor cell destruction by specifically sensitized lymphoid cells. In addition, the techniques developed can be applied to the investigation of mechanisms by which macromolecules induce lymphocyte transformation.

Proposed Course: As outlined above for first year.

Honors and Awards: None

Publications: None

Serial No. NCI-4887
1. Office of the Associate Scientific
Director, Viral Oncology, Etiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Quantitative studies on the transmission of feline oncogenic RNA viruses and selected DNA herpes viruses by certain bloodsucking arthropods.

Previous Serial Number: NCI-4812

Principal Investigator: Dr. George J. Burton

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. Wilna Woods (NCI-4877)
Dr. Ernest Plata (NCI-4828)

Outside NIH:

Dr. K. Maramorosch and Dr. H. Hirumi,
Boyce Thompson Institute, Yonkers, N. Y.
Dr. F. Deinhardt and Dr. L. Falk, Rush-Presbyterian
St. Luke's Medical Center, Chicago, Ill.
Dr. J. Frankel, Life Sciences Inc.
St. Petersburg, Florida

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

1. To function as Project Officer and to advise and guide the Project Director, Dr. Robert G. Fischer, Chairman, Department of Microbiology, University of North Dakota, in the entomological and certain virological aspects of Contract PH43-66-8, having the same project title as given above.

2. To collaborate with those scientists listed above in carrying out specific research projects related to this contract.

3. To assist in procuring animals, arthropods, viruses, and equipment for this contract.

4. To act as entomological consultant to the staff carrying out investigations under this contract.

5. To monitor important experiments at the contractor's laboratory and in cooperating laboratories, and to guide them in developing worthwhile experiments.

6. To advise in the selection and utilization of entomological and other scientific equipment for the project.

7. To keep the Project Director informed of important findings in other laboratories which have a direct bearing on the work of the contract.

8. To act as liaison officer between the Project Director and professional and administrative personnel at NIH, and to assist in processing and expediting requests from the Project Director.

Methods Employed:

In my capacity as Project Officer responsible for the scientific merit and progress of this contract, meetings have been held with the Project Director, the Assistant Project Director, and the Assistant Entomologist, in order to review methods employed and scientific progress on the Contract. Priorities have been set in accordance with the current needs and objectives of the Special Virus Cancer Program. The viruses which were utilized in arthropod transmission studies, including viability within the arthropods selected, were Friend murine leukemia virus, avian reticuloendotheliosis (Twiehaus) virus, feline leukemia virus, and feline sarcoma virus, Marek's disease virus, and herpes saimiri virus. Attempts were made to cultivate Friend virus, MSV and RE(T) virus in *Aedes albopictus* cells. The viability of feline leukemia virus, feline sarcoma virus, and Friend virus was studied in the cat flea, *Ctenocephalides felis* (Bouche). The mosquitoes used in transmission experiments were *Culex pipiens quinquefasciatus* Say, *Culex pipiens pipiens* *Aedes taeniorhynchus* (Wiedemann) Linnaeus, *Anopheles quadrimaculatus* Say, and *Aedes triseriatus* Say. Only one biting fly was studied, the stablefly, *Stomoxys calcitrans* (Linnaeus). The Old World mouse louse, *Polyplax serrata* (Burmester) was also studied as a possible vector of FLV. Contact experiments to determine whether insects could transmit Marek's disease after contacting diseased chickens were also carried out in Tarpon Springs, Florida.

Up to January 1970, the criteria followed for diagnosis of Friend murine leukemia in recipient mice were: a spleen enlargement of 180 mg or more after 21 days, and at least 4 foci in the spleen focus assay. Beginning in

1970 more critical criteria were applied, i.e., a spleen enlargement of at least 275 mg, at least 5 foci in the spleen focus assay, and the XC-Cell Test. Electron microscopic and immunofluorescence studies were also carried out.

Following the study of the extrinsic incubation behavior and viability of each virus within the arthropod, studies were carried out to determine whether the disease could be transmitted from diseased to healthy animals following (a) interrupted feeding activities of the arthropod (mechanical transmission) and (b) normal feeding activities involving transfer of virus via the salivary glands of the arthropod (biological transmission). In the transmission experiments, recipient mice were held from 6 months to one year. Normal mosquito homogenates were also inoculated into mice in order to determine whether false positives could occur as a result of mosquito tissue or secretions alone.

Major Findings:

1. Friend leukemia virus survived only 2-5 days in the tree-hole mosquito, Aedes triseriatus; 1-2 days in the salt-marsh mosquito, Aedes taeniorhynchus, 2-3 days in the malaria mosquito, Anopheles quadrimaculatus, 3 days in the stable fly Stomoxys calcitrans, and 0 days in the cat flea, Ctenocephalides felis.

2. In preliminary experiments, using immunofluoresence, FLV was detected at the end of 21 hours in the infected blood meal taken up by Culex pipiens pipiens, but not at the end of 45 hours, 69 hours, or 90 hours.

3. Herpes saimiri was found to remain viable within S. calcitrans and A. quadrimaculatus, at the end of 6 hours, using a tissue culture assay of white blood cells from the infected blood meal.

4. Detailed electron microscopic examination failed to detect feline leukemia virus in fleas which had fed on FeLV-infected cats, obviously because the titer of FeLV in the donor's blood was too low. When the fleas were fed on a suspension of FeLV-infected material artificially through a skin membrane (titer over 10^7 particles per ml), virus was readily detected in the flea's gut. Similarly, EM examination of cat flea's stomach following artificial feeding on feline sarcoma virus in a titer below 10^7 failed to reveal any virus particles. A valuable contribution concomitant with these studies is that the complete ultrastructure of the cat flea is being worked out, and this has never been done before for any arthropod.

5. C-type particles were found in one kitten which had been injected with an extract of fleas which had had an FeLV-infected blood meal for 24 hours; C-type particles were also found in a kitten which received an extract of fleas which had fed on feline sarcoma virus 48 hours previously.

6. Mechanical transmission of Friend leukemia via interrupted blood meals was accomplished repeatedly with the stable fly. Stomoxys calcitrans,

occasionally with Aedes triseriatus and in only one instance with Anopheles quadrimaculatus.

7. No biological transmission experiments have been positive to date.

Significance to Biomedical Research and the Program of the Institute:

Many bloodsucking arthropods are known to feed alternately on animals (including domestic birds) and man, and such arthropods may be known transmitters of viruses which induce disease in man. Although the murine leukemia viruses and the feline and avian oncogenic viruses are not known to induce diseases in man, it is nevertheless important to learn whether the particular viruses involved may survive, multiply, and possibly be transmitted by arthropods which bite man.

Proposed Course:

It is expected that future studies will involve those which relate directly to man and the possibility of his infection with viruses which may be infective to him, such as herpes saimiri and other herpes viruses.

Honors and Awards: None

Publications: None.

Serial No. NCI-4888
1. Viral Biology Branch
OASDVO, Etiology
2. Molecular Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Molecular Aspects of Viral Oncology and Viral Immunology.

Previous Serial Number: NCI-4818

Principal Investigator: Timothy E. O'Connor

Other Investigators: Dr. Emerson T. Chan, Visiting Scientist
Dr. Paul Ebert (NCI-4876)
Dr. Virginia Dunkel (NCI-4881)

Cooperating Units: None.

Man Years:

Total:	3
Professional:	1
Other:	2

Project Description

Objectives:

1. To explore, at molecular levels, the factors involved in the transfer of specific cellular immunity to previously non-sensitized lymphocytes.

2. To study the biology of animal sarcoma and leukemia viruses to provide defined virus-specific tissue antigens and to develop methods to be applied to isolation of human oncogenic viruses.

Methods Employed:

1. Delayed type hypersensitivity to various well-defined antigens is developed in inbred animals. With appropriate tagged precursor molecules, the molecular species involved in the response of sensitized lymphocytes on exposure to specific antigen may be studied. Passive transfer of specific immunity by whole lymphocytes or defined molecular species is attempted and the efficacy of transfer determined by immunological procedures.

Serial No. NCI-4888

Major Findings:

This study is in an early stage. Preliminary work on the response *in vitro* of sensitized lymphocytes exposed to specific antigen have indicated the probable composition of the molecular species controlling the lymphocyte response. These molecules are currently undergoing biophysical characterization. Cells of a variety of animal species have been transformed by Moloney sarcoma virus or by Schneider-Thielen feline sarcoma virus to provide tumor antigens for study.

Significance to Biomedical Research and the Program of the Institute:

Immunological studies show the importance of cell-mediated immunity in host defense against viral infections and tumor development. In some instances, passive transfer of cell-mediated immunity is possible suggesting value in the control of neoplasms. Theoretical considerations based on vertical transmission of tumor viruses suggest that conventional vaccines may have limited utility in the control of neoplasms induced by such viruses. These investigations on the molecular species involved in passive transfer of cellular immune competence were initiated to provide useful data on alternative safe methods useful for the prevention or prophylaxis of human tumors.

Proposed Course:

The studies on molecular species involved in the passive transfer of cell-mediated immunity will be continued.

Other Activities:

As Advisor to the Associate Scientific Director for Viral Oncology for Molecular Virology and Head, Molecular Virology, in the Office of the Chief, Viral Biology Branch several projects were developed for implementation as research contracts within the Special Virus Cancer Program. Project Officer duties were continued on two contracts, requiring considerable coordination with other contract activities. Duties as an Associate Editor of the Journal of the National Cancer Institute were continued and a term as Associate Editor of Blood was completed.

Honors and Awards:

Elected Vice Chairman of the National Medical and Scientific Affairs Committee of the Leukemia Society of America in January, 1971.

Lectured at the Naval Biological Research Laboratory in Bethesda, at the Institute for Medical Research in Camden, at the Arkansas-Oklahoma Cancer Forum of the American Cancer Society, at Rockefeller University, and at the Second International Congress of Virology in Budapest.

Publications:

Rauscher, F. J. and O'Connor, T. E.: Virology. In Holland, J. F. and Frei, E., III, (Eds.): Cancer Medicine, in press, 1971.

Serial No. NCI-4889
1. Viral Biology Branch
OASDVO, Etiology
2. Microbiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Investigation of Factors which Modify Oncogenic Virus Infection and Oncogenicity both *in vivo* and *in vitro*

Previous Serial Number: NCI-4831

Principal Investigator: Dr. Willie Turner

Other Investigators: Dr. Michael Chirigos (NCI-4875)
Dr. Paul Ebert (NCI-4876)
Dr. John Pearson (NCI-4878)

Cooperating Units: Inside NIH:
Dr. J. Rice, Biology Branch, NCI

Outside NIH:
Dr. G. Spahn, Microbiological Associates, Inc., Walkersville, Maryland
Dr. Peter Gerome, Fort Detrick, Maryland

Man Years:

Total:	1.75
Professional:	1.00
Other:	0.75

Project Description

Objectives:

(A) To further characterize an inhibitor of murine leukemia and sarcoma virus infections.

(B) To study the interaction of lactic acid dehydrogenase virus (LDV) with two strains of murine sarcoma virus, M-MSV and H-MSV, *in vivo*.

(C) To further study poly-d-lysine stimulation of interferon induction by poly(I).poly(C).

Methods Employed:

The inhibitor, STAS, was obtained from silicotungstic acid treated cultures of the JLS-V5 cell line chronically infected with Rauscher murine leukemia virus. Published methods for preparation of inhibitor were followed. Various strains of mice and conventional cell culture and virological techniques were used.

Major Findings:

(A) Studies on STAS, an inhibitor of tumor virus replication: Jasmin and his co-workers reported that STAS markedly inhibited leukemogenesis in mice infected by the Friend murine leukemia virus (FLV) as well as focus-formation in cell cultures infected with M-MSV. To further characterize this inhibitor, we studied STAS modification of MLV-induced infection of mouse embryo fibroblasts (MEF). Infection was monitored by the XC plaque assay. A 99 percent reduction in virus titer was observed in MEF in the presence of 1000 ug of STAS. Potent inhibitory activity was observed with concentrations of STAS as low as 6 ug STAS per ml of culture fluid. Inhibitory activity dropped with time when STAS was added after infection of MEF with MLV. Treatment of cells with STAS 2 or 24 hours prior to virus infection was without effect. Further study showed that residual silicotungstic acid (STA) was the active inhibitor. In an attempt to demonstrate the presence of an inhibitor of leukemogenesis other than STAS, JLS-V5 supernatant culture fluids were processed by the same methods except that other protein-precipitating agents ($ZnSO_4$, PCA, TCA, EtOH, and $(NH_4)_2SO_4$, were substituted for STA. Only $(NH_4)_2SO_4$ preparations showed some activity, suggesting the possible presence of inhibitory activity in JLS-V5 supernatants. XC plaque reduction of 50 percent was considered borderline activity. Thus, a clear demonstration of the presence of a new biological inhibitor of murine leukemia or sarcoma virus replication *in vitro* was not supported in this laboratory.

(B) Potentiation of M-MSV and H-MSV oncogenicity in LDV-infected mice: Steeves and co-workers had reported enhanced FLV-induced spleen focus formation and virus replication in mice previously infected with LDV. Therefore, studies were conducted to determine the effect of LDV on the course of M-MSV and H-MSV infections in mice. LDV-infected mice were challenged with the sarcoma viruses and the tumor incidence and mortality were compared with control animals infected only with M-MSV or H-MSV. A 49 percent reduction in the incidence of tumor regression was observed in LDV-infected animals. A 49 percent increase in mortality occurred. The median survival time(MST) was 61 days less in dually infected animals than in controls infected with M-MSV. In H-MSV infected animals, no tumors in LDV-infected animals regressed in contrast to regression in all animals infected with H-MSV alone. The data show clear enhancement of MSV induced tumors in mice pre-infected with LDV. Simultaneous infection by LDV and MSV did not enhance oncogenic response. Increased titers of H-MSV in extracts of tumors from mice dually infected with H-MSV and LDV were found in comparison with H-MSV controls. The mechanism by which LDV enhances sarcoma virus oncogenicity is unknown.

(C) Enhancement of poly(I).poly(C) induction of interferon by poly-d-lysine: Recent data indicates that poly-d-lysine forms a stoichiometric complex with poly(I).poly(C) with a higher temperature of melting (83°C) than the uncomplexed polynucleotide (63°C). The complex is superior as an interferon inducer *in vivo*. Significant serum interferon titers were attained in Swiss mice during a 24 hour period following intraperitoneal infection of 10-300 ug of the complex at a concentration of 100 to 1000 ug/ml. The serum interferon responses (average and maximum titers) observed in a number of inbred strains of mice given a single 100 ug injection of the complex decreased in the following order: Swiss>DBA/2°C3H>BALB/c>CF-1>AKR>NZB>SJL>NZW, and varied by a factor of about 100 from the most to the least responsive.

Poly-d-lysine of molecular weight$>10^5$, and poly(I).poly(C) prepared from homopolymers of molecular weight 10^5-10^6, are optimally effective in preparing complexes which are effective *in vivo*. Smaller polypeptides of different conformations do not enhance interferon inductions by poly(I). poly(C), and complexes prepared from poly(C) of very high molecular weight are too insoluble for satisfactory application *in vivo*.

An investigation to determine the ability of poly-d-lysine to enhance the antiviral activity of poly(I).poly(C) *in vivo* against murine leukemia and sarcoma viruses as well as tumor cells is being pursued.

Significance to Bio-Medical Research and the Program of the Institute:

Studies accomplished were directed to the evaluation of methods for the more effective control of virus-induced neoplasia using animal model systems. Information gained from such studies could be applied more rapidly and meaningfully in the clinic. The interaction observed between an ordinarily inocuous virus infection and a tumor virus which potentiated oncogenic response in animals is of particular significance in reaching an understanding of mechanisms whereby promoting factors may influence tumor development in man.

Proposed Course:

(A) Studies on the interactions of oncogenic and non-oncogenic viruses will be continued. In addition, the interaction of oncogenic DNA and oncogenic RNA viruses both *in vitro* and *in vivo* will be pursued.

(B) Investigations concerning the immunological relationship between murine Rauscher leukemia virus and Rauscher leukemia virus (HRV) capable of growth in human cells will be initiated. Attempts will be made to "switch on" HRV replication in mouse cells and HRV induced leukomogenic activity in mice by co-infection with non-oncogenic viruses.

(C) The nature of HRV apparent "defectiveness" in mouse cells will be thoroughly investigated with hopes of understanding the nature of "defectiveness" in leukemia-inducing viruses.

Honors and Awards:

None.

Publications:

Turner, W., Ebert, P.S., Bassin, R., Spahn, G., and Chirigos, M.A.: Potentiation of murine sarcoma virus (Harvey) (Moloney) oncogenicity in lactic dehydrogense-elevating virus-infected mice. *Proc. Soc. Exptl. Biol. and Med.* (In press) April issue.

Turner, W., Gibson, W., and Chirigos, M.A.: Enhancement of murine sarcoma virus (Moloney) infection in mice by Guaroa virus. *Cancer Res.* 30: 2645-2651, 1970.

Spahn, G.J., Turner, W., Peters, R.L., and Chirigos, M.A.: Enhancement of Friend leukemia virus infection in mice by Guaroa virus: quantitation and action by various oncogenic and non-oncogenic viruses. *Applied Micro.* 20: 551-554, 1970.

SUMMARY REPORT

VIRAL CARCINOGENESIS BRANCH

July 1, 1970 June 30, 1971

During the past year the comprehensive and highly integrated intramural and contract research programs of the VCB were reoriented to test the validity of the new oncogene theory of cancer. This unitary theory (first proposed by VCB investigators in 1969) postulated that most, if not all, types of cancer are specified by inherited RNA tumor virus oncogenes which like all other gene operons are controlled by other regulatory genes.

Cancer which in natural (feral) species generally occurs after the reproductive period is viewed as the result of breakdowns in appropriate regulation brought on by (1) endogenous factors such as mutant defective genes, hormonal imbalances, immunological deficiencies, and simply senescence; and (2) exogenous factors such as environmental carcinogens. One of the chief objectives, then, is to discover the molecular and biochemical mechanisms by which cells regulate oncogenes in the hope of finding ways to postpone neoplastic change.

Numerous isolates of the C-type RNA viruses are now established in laboratory systems from three classes of vertebrates (snakes, birds and mammals), and from three orders and four species of mammals. Since current evidence indicates that RNA viruses in these vertebrates do not depend on horizontal transmission, but on genome inheritance, we postulate further that the arrangement (integration) of the RNA viral genomes with cell genomes must have been already well established in the common ancestors of these different vertebrates, thus an arrangement that probably existed more than 300 million years ago. The biochemical similarities exhibited by the major polypeptide group-specific (gs) antigens of the C-type RNA viruses of the three classes of vertebrates, which when assembled constitute 30% of the mass of the C-type particle, provide additional contemporary evidence of common phylogenetic relationships and attest further to a common ancestry and a profound evolutionary linkage to the species in which they are found. It is very unlikely that man, who experiences cancers very similar to those of the other mammals, should have escaped involvement with these "built in" RNA oncogenes despite his long span of life and the longer periods during which the oncogenes must be maintained in repressed state.

More specific recent support for the oncogene theory.

In 1971 the heuristic value of the oncogene theory was proved in terms of the many newly launched research ventures stimulated by it, particularly in molecular biology, *in vitro* carcinogenesis, and immunogenetics. Although the theory still requires definitive proof, considerable confirmatory evidence in support of it was brought forth in FY 1971 by investigators outside the VCB as well as those in it. The landmark discovery of the reverse RNA transcriptase by Temin and Baltimore supplied a clearcut mechanism by which RNA genomes could be integrated with host cell DNA; indeed vertical transmission and inheritance of the RNA genomes are implicit in the new protovirus theory of Temin. Similarly, recent reports by Vogt and Hanafusa describe endogenous RNA virus "helper" factors in normal embryonic cells of chickens; others, including Meier, Lilly, Pincus and Hartley, Payne and Chubb, working with inbred mice and chickens, elucidated both dominant and autosomal recessive host genes which clearly regulate and control the various antigenic, infectious and oncogenic expressions of the endogenous RNA tumor virus genomes. Recently, also, the Russian scientist Abelev and his associates in a series of reports confirmed previous findings in VCB that gs antigen subunits of the RNA virus are prevalent in embryonic and tumorous tissues of all strains of mice, even those having maximum genotypic resistance to cancer and RNA virus expressions. Finally, intramural and contract supported investigators working on highly targeted VCB programs have contributed significantly in elucidating (1) the natural behavior of the RNA tumor virus genomes in mice, chickens, hamsters, cats and rats; (2) the profound effects of host genotypes on cancer and virus activity; and (3) the biochemical and immunological specificities of the independent virogene and oncogene expressions specified by the RNA virus genomes, including those which occur "spontaneously" and those which are induced by various carcinogenic agents.

The most significant contributions of the VCB program in Fiscal Year 1971 can be briefly highlighted as follows:

1.0 Properties and natural prevalence of the group-specific (gs) antigens of the C-type RNA virus genome.

1.1 The gs antigens of the RNA myxoviruses represent perhaps the best studied of viral products; they provide the basic marker used for group classifications of the numerous influenza and parainfluenza viruses of man and animals. Similar gs antigens, first reported in 1964 by VCB scientists, have been demonstrated in the RNA viruses of chickens, mice, cats, hamsters, rats, and snakes. These antigens which have been studied intensively by some of the top biochemists and immunologists in the world, provide a diagnostic marker (handle) for the RNA tumor viruses which not only makes it possible to demonstrate the presence of the genome in cells and tumors free of infectious virus, but has increased at least 100-fold our knowledge of the natural distribution of the RNA tumor virus genome.

1.2 Isolation, purification of gs antigen and preparation of specific antibody to it: In FY 1971 VCB investigators Drs. Gilden, Oroszlan and Kelloff succeeded in isolating and highly purifying the gs antigens of mouse, cat, hamster and rat, following which monospecific antisera to the major gs-1 component were prepared in guinea pigs and rats. When purified by isoelectric focusing, the gs antigens from all 4 mammals were found to be stable homogenous molecules with molecular weights of 20,000 to 30,000; however, when assembled they found that the gs antigen molecules make up at least 30% of the total virus particle. Gilden et al. confirmed the separate reactivities of the major gs-1 (species specific) and gs-3 (interspecies) determinants; however he subsequently showed that both determinants were located on the same molecule.

1.3 Universal prevalence of gs antigens: In the meantime, Huebner, Sarma, Kelloff, Gilden, Meier, Whitmire, Peters, and Gardner, with the use of rat, guinea pig and hamster antisera to gs antigens, reported that gs antigens were commonly detectable in virtually all embryonic tissues of mice, certain rapidly replicating postnatal tissues, and many if not most spontaneous and chemically induced cancers of mice. Sarma and associates, using hamster sera and rabbit sera (from Dr. David Allen) specific for avian RNA virus gs antigens, similarly demonstrated expressions of gs antigens in all avian embryos from 4 to 20 days *in ovo* including those derived from RIF-free flocks known to be free of infectious RNA virus. Similar but less extensive studies have indicated widespread distributions of gs antigen expressions in embryos and tumors of cats and hamsters as well.

1.4 Immunological tolerance to gs antigens: These direct demonstrations of RNA C-type virus expressions in prenatal tissues were followed up by studies which revealed that all mice, hamsters, cats, and nearly all chickens were inherently incapable of producing antibody to the gs antigens of their isologous species. The fact that most members of these species could produce antibodies to gs antigens in heterologous species and to envelope antigens of their own species showed not only that the antigens were antigenic but that the animals were immunologically competent. This classic manifestation of immunological tolerance provided solid support for our hypothesis that the universally distributed embryonic gs antigen expressions probably play important roles in the development and differentiation of embryonic tissues. This concept leads logically to the speculation that the RNA genome activities may for the most part be beneficial perhaps by helping to regulate cell replication; viewed in this way, the development of cancer then results from an unfortunate breakdown in normal regulating mechanisms induced by endogenous or exogenous factors. Cancers therefore would appear as abortive efforts to produce a genetically identical twin at an inappropriate time and place, thus accounting for the fact that the body generally recognizes and accepts the tumors as "self."

1.5 Activation of gs antigens concomitantly with induction of chemically induced tumors in mice: Previous reports by VCB investigators of the activation of complement-fixing gs antigens in subcutaneous fibrosarcomas induced in mice by 3MC were confirmed in FY 1971 by Whitmire and associates in 16 strains of inbred and outbred laboratory mice. Complement-fixing antigens reactive with anti-gs rat antisera were demonstrated in most of the fibrosarcomas when tested with broadly reactive antisera. When tested with specific antisera to gs-1, the distribution of positives in the inbred mice varied from 0% to 100% depending on the genotypes of the different strains. The k, s and q alleles were associated with high incidences of gs-1 antigen expression, while the b and d alleles were associated with lower incidences. Since adjacent normal mesenchymal tissues were negative for antigen, the switch on of gs antigen in tumors was attributed to derepression of the RNA virogenes concomitant with tumor development.

1.6 In vitro activation of gs antigen (and infectious virus) in mouse, hamster and rat fibroblasts: That individual mouse cells (C3H fibroblasts) grown in culture can switch on oncogenes was first observed by Earle and his associates; subsequently Evans, Hartley *et al.* reported that most such cells were "switched on" for gs antigen and infectious expressions of RNA virus, and in 1970 Aaronson, Hartley and Todaro made similar observations in additional cultured mouse cells.

In FY 1971, Freeman, Hartley, Gardner, Officer and Kelloff observed spontaneous and chemically induced switch on of murine RNA viruses in wild (feral) mouse cells; recently, also, of hamster RNA viruses in 5 of 6 hamster cell lines transformed by 3MC and fractions of tobacco smoke.

Finally, Rhim and his associates discovered a new antigen having the properties of gs antigens in rat cells transformed in early subcultures by polyoma virus and in the normal rat cell lines when carried beyond 60 subcultures.

We concluded from these findings that mouse, hamster and rat cells grown *in vitro* contain "switched off" RNA virus genomes that are subject to derepression not only by many different chemical and DNA virus carcinogens, but also as a consequence of long term culture. Such observations not only make less likely the contention that mesenchymal tumor cells *in vivo* depend for their viral expressions on the migration of virus information from other areas in the body such as the hematopoietic tissues, but furnish cogent reasons for assuming that the oncogenic and virogenic events were indeed induced *in situ*.

2.0 Oncogenes of C-type RNA infectious viruses as determinants of accelerated cell transformation by carcinogenic chemicals and DNA tumor viruses: In a series of recent reports Freeman, Rhim and Huebner and their associates described greatly accelerated transformations induced by various carcinogenic chemicals in rat, mouse and hamster cells when such cells were superinfected with various mouse and hamster non-transforming C-type RNA viruses. The normal cells treated with chemicals alone or virus alone failed to transform and could not be transplanted in isologous newborn hosts; however the infected cells treated with carcinogens readily transformed and in most instances were transplanted successfully in newborn isologous hosts.

Thus cells of 3 species cultured *in vitro*, carrying one of four strains of RNA tumor viruses (RLV, CF-1, AKR, HaLV) and treated with at least 8 different carcinogenic chemical preparations, in every case led to the development of tranformed foci, continuous neoplastic cell lines and, with only one exception, tumors in isologous hosts. The transformations occurred rapidly within 9 to 45 days after 7 to 14 days of exposure to the chemicals in the media. Dosages as low as 0.01 ugm of MC and DMBA produced rapid transformation; thus providing at least 100-fold range for carcinogenic assay below the toxicity levels of the chemicals. The high predictability achieved with many different combinations of viruses, cells and chemicals clearly suggested that the added RNA viral genomes provided numerous additional oncogene determinants which, when derepressed by the chemicals, accounted for the accelerated neoplastic changes observed.

3.0 RNA dependent DNA polymerase; enzymatic and antigenic properties: VCB contract supported scientists were among the first to extend Temin's and Baltimore's observations on the reverse transcriptase. Green and associates showed that the entire RNA genome of avian, mouse and cat viruses was copied by the polymerase as small DNA molecules and that the DNA dependent as well as the RNA dependent polymerases were inhibited independently by various rifampicin derivatives. The latter also inhibited RNA-DNA hybrids while duplex DNA's were inhibited by actinomycin derivatives. These observations were interpreted as providing potential leads to a rational approach to chemotherapy of cancer.

Current studies in cooperation with Dr. Green will explore the effects of rifampicin specifically on endogenous oncogene expressions in vitro. Inhibitors specifically directed against the RNA polymerases responsible for assembly of the virus particle may not have specific activities in relation to transformation or tumor induction since the oncogene expressions are independent of the virogene expressions. Since most cancer must be due to endogenous rather than exogenous viruses, answers to the question of specificity inhibitor action are critically needed.

3.1 Specific antibody to RNA dependent DNA polymerases: Equally as exciting were the findings of Gilden and Hatanaka (Flow Laboratories, Inc.) who purified the cat virus polymerase and with it produced monovalent antibody which inhibited the activity of the mouse, cat, hamster and rat virus polymerases but not that of the avian viruses. Thus the polymerase represents an additional group-specific interspecies antigen protein of the mammalian viruses; and the ability to specifically inhibit polymerase activity should make it possible to determine its role in spontaneous and carcinogen-induced cancers, both of which we postulate are the product of endogenous oncogenes.

4.0 Host gene controls of RNA tumor virus expressions: One of the spin-offs of the inherited oncogene theory was increased interest in studies of genetic and moelcular mechanisms by which host genes influence expressions of the postulated endogenous virogenes and oncogenes. Meier et al., who in FY 1971 provided much of the natural history data on the universality of the C-type RNA genomes and lack of horizontal spread of virus in inbred mice at The Jackson Laboratory, also observed large and well defined influences of strain genotypes on predisposition or resistance to the development of spontaneous cancer, to susceptibility to environmental carcinogens, and to various independent expressions of the RNA tumor viruses.

Cross-breeding and other experiments have revealed the importance of a variety of dominant and recessive alleles as determinants of RNA virus and tumor expression. Breeding programs are well underway which indicate that by genetic manipulation, high cancer (lymphoma) strains can be converted to resistant strains and vice versa. Current experiments are designed to determine the number, mode of inheritance, linkage relationships and chromosome location of the host genes which regulate oncogene and virogene expressions.

4.1 Studies of host gene controls in vitro: Similarly, Hartley, Rowe and Pincus in characterizing embryo cells derived from 21 strains of mice according to sensitivity to N- or B-tropic murine leukemia viruses, found that all cells were similar to either the N-type or B-type. The patterns of sensitivity of N-type x B-type F1 hybrids indicated dominance of resistance to both types of virus, and embryos from back-cross experiments suggested that a single locus (N-B) is the primary determinant of the host cell response patterns; subsequently the N-B locus was found to be identical with the FV1 locus which controls sensitivity to Friend leukemia virus.

The numerous host genes that have been found to control RNA virus expressions and the discovery that the "resistance" allele on the N-B locus controlling wild type infectious RNA virus replication not only supports the inherited oncogene theory, but provides evidence of built-in natural host gene systems designed through long evolutionary association to control the RNA viral genome. This is also part of the natural inheritance of mice. Similar host controls of cancer and RNA virus have been described for chickens. It would seem inevitable therefore that similar host genes and endogenous RNA genomes will be found in the cells of most or all other vertebrates.

5.0 Cat leukemia (FeLV) and cat sarcoma (FSV) viruses: detection and natural history: FeLV and FSV have special importance because infectious virus is rather prevalent in tumored cats; the virus is found in cat salivary secretions and is known to grow well in human cells in culture. Consequently VCB investigators (Sarma, Gardner, Riggs, Oshiro, Schneider and Arnstein) have been much involved in developing and applying sensitive detection methods such as the new COCAL test for FeLV, focus-forming tests for FSV, fluorescent antibody (FA), complement-fixation (CF), neutralization and other serological tests for antigen and antibodies. Serological studies by Riggs, Oshiro and Sarma have shown that sera from 600 veterinarians show no evidence of specific FA or neutralizing antibodies to FeLV; thus despite maximum exposure to sick and cancerous cats, veterinarians apparently are not susceptible to infection with cat viruses.

5.1 Epidemiological studies of cats as a cancer risk factor: Controlled epidemiological studies designed to examine human exposure to cats as a factor in human cancer cases in Los Angeles by Gardner and Hanes of the University of Southern California showed no excess exposures to cats in the households of several hundreds of leukemia, sarcoma and carcinoma cases as compared to comparable control households. In fact 80% of the cancer and control households had no household exposure to cats during the previous 10 years.

A similar survey in Northern California by Schneider failed also to show that household exposure to leukemic cats increased the incidence of cancer in the exposed persons as compared to control households without such exposure.

These studies of course do not eliminate the possibility that human cancer might occasionally result from a cat virus source, but they suggest that cat virus is unlikely to account for detectable amounts of human cancer. The study in Northern California will be carried on as a prospective study in order to increase numbers and more conclusive interpretation.

6.0 Etiological and epidemiological studies of human cancer.

6.1 Search for evidence of C-type RNA virus expressions: The search for C-type RNA viruses in human cancers at the University of Southern California were no more successful in FY 1971 than in previous years. Electron micrographic examinations of hundreds of human tumors including all available types of leukemia and solid cancers, and of embryo and tumor cell cultures, were entirely negative for C- and B-type particles. The tumors were acquired and examined at USC and the cultures were produced at the Naval Biological Laboratory under Dr. Nelson-Rees.

Since there are no currently extant human cell cultures carrying C-type particles, and since infectious virus has proved equally elusive, the VCB's future efforts will be focused on attempts to demonstrate antigens in human tumor and embryo cells which might be expected to cross react with one or more of the interspecies antigens demonstrated in mouse, hamster, cat and rat C-type viruses; we plan to look for evidence of all types of antigens specified by the known RNA viruses including: group-specific antigens 1, 3 and other postulated interspecies antigens; the cell surface antigens specified by the RNA genome, variously described as GSA (Aoki) and TSTA (Sjogren and Pasternak); and viral envelope antigens.

6.2 Serological data: Recent attempts to demonstrate cross-reacting antigens by complement-fixation in extracts of human tumors, utilizing broadly reactive anti-gs antisera produced in Fischer rats carrying MSV-induced tumors or in rats hyperimmunized with extracts of the MSV rat tumors, yielded many positive human tumor and embryo tissue preparations; however the reactive antigenic moieties so far have been found to be ether resistant and associated with sedimentable cell membranes. Surveys of human cancer patient sera have yielded a few which react at low serum dilutions with the sedimentable antigens and the patterns of serum reactivity are almost exactly similar to that provided by the rat sera. Although the antigens detected are clearly not related to the gs antigens found in the mammalian RNA tumor viruses, they appear to be shared widely and thus are interesting enough to be characterized and identified. It is possible that they will prove to be similar to the carcino-embryonic antigens described by Gold, Uriel, Grabar and others.

New and more sensitive autoradiographic serological test systems developed by Gilden's group, in addition to CF, FA and gel diffusion, will be combined with absorption studies and utilized in an exhaustive study of human tumors for antigens related to those now demonstrable in the RNA viruses and tumors of mice, cats, hamsters and rats.

In order to adequately prepare for such studies, numerous human tumors and sera from cancer patients have been collected and frozen for future studies; cell lines have been derived from many tumors and some of these are being subjected to intensive studies as described below.

6.3 Studies of human tumor cells in vitro: Having established several human sarcoma cell lines which have retained their sarcomatous characteristics, McAllister and his associates at Children's Hospital of Los Angeles have attempted to rescue the sarcoma genome by superinfecting them with murine and cat viruses; these efforts have been unsuccessful despite the fact that the viruses replicated in the human cells. In cooperation with Klement, Nicolson and Gardner, McAllister is seeking evidence of phenotypic modifications in the mouse and cat viruses replicating in the human tumor cells. Current tests suggest modification of the envelope and host range but not of viral gs antigen and RNA moieties; tests of the specificities of the viral RNA polymerases are underway. Serial subcultures of the tumor cells, both infected and non-infected, are being continued indefinitely in anticipation that, like mouse, rat and hamster cells, human cells that grow continuously in vitro will eventually derepress and yield expressions of the postulated human RNA virus genome. At various subcultures the human sarcoma cells were transplanted into cat fetuses in which tumors developed having histological appearances identical with that of the original tumors seen years before in the patients; and chromosome histograms confirmed the human characteristics of the tumor cells. A similar in vivo human tumor cell growth system in the mouse developed recently by Stanbridge and Hayflick which utilizes ALS to block rejection of the human tumor cells provides a much more available and simpler system for studies of genome rescue of these and other human tumors.

6.4 Transformations of human cells with polyoma virus: In preliminary attempts it appears that Dr. Rhim in collaboration with Dr. Takemoto (NIAID) has succeeded in transforming human cells (WI-38) with large doses of polyoma virus. The cells contain polyoma T antigen but no polyoma viral antigen. Since rat and hamster cells transformed by polyoma virus develop new antigens which are distinct from known polyoma specified antigens, but which react with MSV rat antisera, related antigens will also be sought in the human polyoma transformed cells as well.

6.5 Epidemiological and ecological studies of cancer distributions in Los Angeles: A comprehensive cancer surveillance program designed to provide a "now" registry of all cancer patients in Los Angeles County was planned in FY 1971; as this is funded in 1972, Drs. Henderson, Hanes and Gardner, in cooperation with NCI's demography and epidemiology groups, and with Shimkin (University of California, San Diego) and Winkelstein (University of California, Berkeley), plan to utilize information eventually to be made available on 20,000+ annual cancer cases in Los Angeles in order to mount field studies designed to answer specific questions concerning the etiology of cancer. Examples include questions about effects of maternal age on cancer in offspring and of course the effects of industrial, food-borne and air-borne carcinogens. In support of the latter program, Bryan and Gordon have underway long term studies characterizing smog constituents in 4 different ecologic areas in Los Angeles. Freeman, Rhim, Rasheed and Gardner have already initiated studies of smog residue and tobacco smoke fractions in cell culture systems; preliminary results now in progress and in press indicate that such materials have relatively strong carcinogenic activities (vide supra). Future studies will be focused on tests of chemically defined fractions of known environmental residues and on such additional assays as may be indicated by the results of epidemiological and enviromental studies in the Los Angeles ecology.

Serial Number NCI-4922
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Laboratory and Field Studies of the Role of C-type RNA Viruses in Human and Animal Cancer

Previous Serial Number: NCI-4911

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Dr. D. Hammond, Childrens Hospital of Los Angeles
Dr. W. Nelson-Rees, Naval Biomedical Research Laboratory, Oakland, Calif.
Dr. Adeline Hackett, Naval Biomedical Research Laboratory
Dr. Edwin Lennette, California State Department of Public Health, Berkeley, Calif.
Dr. John Riggs, Calif. State Dept. of Public Health
Dr. Lyndon Oshiro, Calif. State Dept. of Public Health
Dr. Leonard Hayflick, Stanford University, Stanford, Calif.
Dr. Lloyd Old, Sloan-Kettering Institute for Cancer Research, New York, N.Y.

Man Years:

Total:	22.0
Professional:	2.0
Other:	20.0

Project Description

Refer to Summary, VCB, NCI

Honors and Awards: (Dr. Robert J. Huebner)

Kimble Methodology Award, 1970
Rockefeller Public Service Award, 1970
Honorary Degree, Doctor of Science, University of Parma, 1970

Invited Speaker:

Scientific Research Society of America, Atlanta, Ga., Sept. 1970.
Conference on Immunological Tolerance to Microbial Antigens, New York Academy of Sciences, N. Y., Sept. 1970.
Yale University, Dept. of Molecular Biophysics and Biochemistry, Seminar, New Haven, Conn., Oct. 1970.
The New York Cancer Society, Lecture, New York, N. Y., Nov. 1970.
Academy of Medicine of Cincinnati, Lecture, Cincinnati, Ohio, Nov. 1970.
American Academy of Dermatology, Lecture, Chicago, Ill., Dec. 1970.
University of Parma, Seminars, Parma, Italy, Dec. 1970.
The Milton S. Hershey Medical Center, Seminar, Hershey, Pa., Jan. 1971.
University of Pennsylvania, Dept. of Microbiology Seminars, Phila., Pa. Feb. 1971.
24th Annual Symposium on Fundamental Cancer Research, M. D. Anderson Hospital, Houston, Texas, Mar. 1971.
University of Chicago Dept. of Medicine, Chicago, Ill., March 1971.
Conference on RNA Viruses and Host Genome in Oncogenesis, Speaker, Amsterdam, The Netherlands, May 1971.

Publications:

Huebner, R.J., Kelloff, G. J., Sarma, P.S., Lane, W.T., Turner, H.C., Gilden, R.V., Oroszlan, S., Meier, H., Myers, D.D., and Peters, R.L.: Group-specific antigen expression during embryogenesis of the genome of the C-type RNA tumor virus: implications for ontogenesis and oncogenesis. Proc. Nat. Acad. Sci. 67: 366-376, 1970.

Huebner, R.J., and Igel, H.J.: Immunological tolerance to gs antigens as evidence for vertical transmission of noninfectious C-type RNA virus genomes. In Pollard, M. (Ed.): Perspectives in Virology VII. New York, Academic Press, 1971, pp. 55-71.

Mellors, R.C., Shirai, T., Aoki, T., Huebner, R.J., and Krawczynski, K.: Wild-type Gross leukemia virus and the pathogenesis of the glomerulonephritis of New Zealand mice. J. Exp. Med. 133: 113, 1971.

Huebner, R.J., Todaro, G.J., Sarma, P., Hartley, J.W., Freeman, A.E., Peters, R.L., Whitmire, C.E., Meier, H., and Gilden, R.V.: "Switched off" vertically transmitted C-type RNA tumor viruses as determinants of spontaneous and induced cancer: a new hypothesis of viral carcinogenesis. In Defectiveness, Rescue and Stimulation of Oncogenic Viruses. Editions du Centre National de la Recherche Scientifique, Paris, 1970, pp. 33-57.

Huebner, R.J.: Identification of leukemogenic viruses: specifications for vertically transmitted, mostly "switched off" RNA tumor viruses as determinants of the generality of cancer. In Dutcher, R.M. (Ed.): Comparative Leukemia Research 1969. Bibl. Haemat., No. 36, Basel, Munchen, and New York, Karger, 1970.

Serial No. NCI 4924
1. Viral Carcinogenesis Branch
   OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) Immunological Comparisons of Murine Sarcoma Viruses Grown in Mouse and Human Cells.

(B) Purification of and Production of Specific Antibody to the Envelope Antigens of the Mammalian C-type Viruses.

(C) Production of Synkaryons for Use in Mammalian C-type Virus Studies.

(D) Completion of Comprehensive Study on the Hamster C-type Virus (HaLV).

Previous Serial Number: NCI 4912

Principal Investigator: Dr. Gary J. Kelloff

Other Investigators: Dr. Raymond V. Gilden, Flow Laboratories, Inc., Rockville, Maryland.
Dr. Cedric Long, Flow Laboratories, Inc., Rockville, Maryland
Dr. Stephen Oroszlan, Flow Laboratories, Inc., Rockville, Maryland

Cooperating Units: Inside NIH:

Dr. Robert Huebner, VCB, NCI
Mr. William T. Lane, VCB, NCI

Outside NIH:

Dr. Aaron Freeman, Microbiological Associates, Inc., Bethesda, Maryland

Man Years:

Total: 1.0
Professional: 1.0
Other: 0.0

Project Description

Objectives:

(A) (1) To study in detail the immunological and virological properties of the Kirsten strain of murine sarcoma virus (Ki-MSV) grown on mouse cells to Ki-MSV grown on human cells (Ki-MSV/909): (2) Production of neutralizing antibody to both viruses: (3) Investigation of Ki-MSV/909 for heterogenecity: (4) Envelope antigen purification of both viruses.

(B) (1) Production of neutralizing antibody to the mammalian C-type RNA viruses: (2) Separation and purification of the envelope antigens of the mammalian C-type viruses: (3) Production of unispecific antibody to the envelope antigens of these viruses: (4) Study of the natural incidence of C-type virus envelope antigen and envelope antibody in the mammalian host and its relationship to spontaneously occurring neoplasms.

(C) (1) Production of synkaryons to examine the possibility of derepression of latent virus: (2) Establishment of synkaryons of several species in search of marker chromosomes needed for C-type virus replication: (3) Use of synkaryons as susceptible cells for virus isolation.

(D) (1) To investigate the virologic and immunologic properties of the hamster specific sarcoma and helper viruses previously described: (2) To determine the natural expression of the C-type virus in various ages and in various tissues of the normal hamster, and in hamsters bearing tumors of various etiologies: (3) Studies on co-carcinogenesis of the hamster leukemia virus (HaLV) and chemical carcinogens.

Methods Employed and Major Findings:

(A) Continuous cell lines shedding Ki-MSV/3T3 and Ki-MSV/909 have been derived respectively from cloned foci of BALB/c 3T3 cells and a human skin fibroblast line 909 (kindly supplied by Dr. V. Klement). From supernatant fluids, virus is banded on sucrose density gradients and is used for immunization of guinea pigs for production of neutralizing antisera. Standard tissue culture methods are used for production of stock virus and for virus titrations. Virus neutralization tests are done by the focus induction or inhibition of focus reduction technique. Studies of the structural polypeptides of the two viruses are done primarily by acrylamide gel electrophoresis of radioactively labelled virus. Radioactive isotopes used are primarily H^3 and C^{14} amino acids and H^3 and C^{14} labelled glucosamine. Immunologic tests also include complement-fixation and immunodiffusion.

To compare quantitatively the differences in host range of Ki-MSV/3T3 and Ki-MSV/909, banded preparations of the two viruses were made and quantitated by protein determinations and complement-fixation using antisera to the murine group-specific antigen. Viral preparations of Ki-MSV/3T3 with infectivity titers of 10^5 on homologous cells (B/c 3T3) showed no activity on heterologous cells (909); conversely preparations of Ki-MSV/909 with infectivity titers of 10^3 on 909 were not active on B/c 3T3 cells. The use of DEAE dextran did not alter these results. This result suggested that preparation of neutralizing antisera to each of these two agents might be fruitful. Antisera have been obtained with neutralizing endpoints as high as 1:5000 against homologous virus and there does not appear to be any significant cross-neutralizing activity with these antisera made to either virus. Also, antisera made to normal B/c 3T3 and 909 cells fail to neutralize the homologous viruses grown in these cells. Lack of a good human cell for focus assays has made the studies unduly cumbersome. However, rat embryo fibroblasts have recently been used to titrate Ki-MSV/909 and the results have been much more satisfactory. Inhibition of neutralization using banded Ki-MSV/3T3 and Ki-MSV/909 have corroborated this lack of cross-reactivity at a level of at least 100 - 1000 fold difference in inhibitory antigen. These inhibition studies have suggested that a useful general assay for envelope antigen activity for C-type viruses can be developed. This is discussed below. Acrylamide gel electrophoresis studies of the structural proteins (polypeptides) of the two virus preparations are still preliminary and no definite statements can be made at present. Detailed studies with H^3 and C^{14} labelled glucosamine are planned to fully investigate the difference in the envelope antigens of the two viruses.

(B) Continuous cell lines shedding AKR, Rauscher leukemia virus (RLV), hamster leukemia virus (HaLV) and feline leukemia virus (FeLV) have been produced. From supernatant fluids, virus is banded on sucrose density gradients and is used for immunization of rabbits. Standard tissue culture methods are used and virus neutralization tests are done by the focus reduction technique. Radioactive labelling techniques are as described above.

Production of large standard pools of MSV(AKR), MSV(RLV), Ki-MSV, and M-MSV(HaLV)G for use in the focus reduction test are almost completed. Neutralizing sera from test bleeds of some of the immunized rabbits have recently yielded usable neutralizing sera, and establishment of standard curves for these sera is now in progress. Preliminary studies show that neutralizing endpoints for these sera show sharp endpoints over a two-to-four fold dilution range suggesting that a very usable assay for envelope antigen activity can be developed by inhibition of focus reduction. Using sucrose banded Ki-MSV as inhibiting antigen, it has been found that about 0.5 CF units (as measured by gs antisera) of this antigen will almost completely inhibit neutralization of the standard virus dose (100 FFU). Separation and purification of the envelope antigens by acrylamide gel electrophoresis is just being initiated. The initial methods utilized will be similar to those already published for the avian C-type viruses (Duesberg, 1970; Bolognesi and Bauer, 1970).

(C) Enzyme dependent cells have been produced by passage of these cells in Budr or thioguanine. The resulting cell lines have been shown by isotopic enzyme methods to be deficient in thymadine kinase (TK) and hypoxanthene-guanine phosphoriboxyl transprose (HGPRT), respectively. These cells have been fused by inactivated Sendai and cultured in selective media under conditions that both parental cell types can be selected against leaving only the hybrid cells as survivors. Standard methods for virus isolation and titrations have been used. Complement-fixation tests with antisera to the murine and hamster C-type virus have been utilized. Electron microscopic examination has been performed according to published methods. Examination of chromosomes and characterization of enzymes by iso-electrofocusing techniques are in progress.

Hybrids of HT-1 cells (carrying the defective MSV genome) have been made with BALB/c 3T3 cells. The resulting hybrid contained defective MSV genome rescuable by helper virus, but no evidence of derepression of the presumed indigenous C-type RNA virus of the mouse has yet been detected. Hybrids made with leukemia virus infected B/c 3T3 cells have yielded focus-forming virus. Many studies are currently in progress with these hybrids and many other hybrid lines have been made or are currently being made.

(D) Standard tissue culture methods for virus growth and titration were used. Cloning experiments of foci or of normal cells were performed with neutralizing antisera in the media present at all times to enhance the changes of measuring only input virus, and isolating non-producer foci and clones of cells shedding only helper virus. Antisera has been prepared against the purified gs antigen of the hamster C-type viruses. Neutralizing antisera has been prepared in rabbits as described above. Immunological tests include complement-fixation, immunodiffusion and virus neutralization. Antigens tested were 20% extracts of tissues from normal and tumor-bearing hamsters and from in vitro preparations. Screening of these tissues were also performed by electron microscopic examination. The structural polypeptides of the hamster C-type virus have been separated and purified by acrylamide gel electrophoresis similar to previous studies on the murine C-type virus (Oroszlan et al.). Chemical co-carcinogenesis studies were done in collaboration with Dr. Aaron Freeman.

We had established previously that three hamster sarcoma viruses derived from hamster tumors induced by murine sarcoma viruses share at least one envelope antigen and an internal ether resistant antigen with a hamster helper virus (HaLV) isolated from preparations of one of these viruses [M-MSV(HaLV)O]. Further, an indigenous hamster C-type virus was also shown to share this internal ether resistant (gs) antigen, which led us to postulate that the three hamster sarcoma viruses and pseudotypes have their host range and antigenic specificity supplied by the indigenous hamster virus. Preparations of the other two hamster sarcoma viruses had not shown any helper virus activity by endpoint dilution techniques, therefore cloning experiments were made to examine these virus pools for helper virus. Twenty clones of normal cells were obtained from plates infected with 5-10 focus forming units (FFU) of the two sarcoma viruses. After one re-cloning, these clones were passaged and screened in the complement-fixation test with anti-hamster gs antisera. Two clones from Ki-MSV(HaLV) and two clones from M-MSV(HaLV) were found to be positive. Cell lines derived from these clones have not yielded focus forming virus, but when co-cultivated with HT-1 cells, the resulting harvests have yielded focus forming virus on hamster embryo fibroblasts. We have assumed then that helper virus is present in the stocks of these sarcoma viruses, but it is not necessarily found in excess of the latter. By similar techniques non-producer clones were derived from the three hamster virus stocks. These clones were found to be inactive in complement-fixation tests and no detectable virus by EM, H^3 labelling or direct isolation attempts, but focus forming virus was rescuable by co-cultivation with leukemia virus.

Since in vivo rescue of HaLV has been obtained with all three input murine sarcoma viruses, attempts were made to achieve this in vitro. A murine sarcoma virus, M-MSV(RLV), capable of growth on hamster cells, was kindly supplied by Dr. R.C. Ting. This virus contains only murine C-type virus antigens, therefore presence of hamster virus antigens in input hamster cells could be assumed to originate in these cells. Studies of eight clones of hamster cells transformed by this MSV failed to show any reactivity with hamster gs antisera.

All tissues from 3 strains of hamsters (Graffi, LSH and NIH), ranging in age from 1 month to 25 months, were tested in complement-fixation, by electron microscopy, and by direct isolation techniques for the presence of hamster C-type virus. No positives were found nor was any evidence of hamster C-type virus found in hamster tumors induced by DNA viruses. However, gs antigen has been found consistently in 8-9 day Graffi embryos. Tumors induced by chemically transformed hamster embryo fibroblasts have been shown to contain an agent similar to HaLV. Tumor transplant lines obtained from Dr. A. Nahmias and Dr. M. Finkel have been shown to be positive by these methods.

Significance to Biomedical Research and the Program of the Institute:

(A) The earlier published findings of rescue of hamster C-type virus from hamster tumors induced by murine sarcoma viruses suggested that in vitro studies of murine sarcoma virus grown in heterologous species should be done. We have since found that hamster embryo fibroblasts infected singly with a defective murine sarcoma genome show no evidence of hamster C-type virus activity. We can assume then that it is likely that the reported changes of Ki-MSV grown in human cells represent host cell induced modifications and do not in any way represent isolation of a new agent (this is confirmed by the presence of the murine gs antigen in Ki-MSV/909). However, the recent findings of Hanafusa of isolation of a presumed indigenous avian C-type virus in leukosis virus-infected cells and the still unresolved origin of RSV(O) suggest that the stocks of Ki-MSV/909 should be examined for the presence of a low titered new isolate.

The question of the host cell contribution to the antigenic structure of the C-type viruses is an important problem to resolve, since many investigators are involved in studies of C-type viruses grown in heterologous cells. Further, the basic question of what antigen is actually involved in the neutralization reaction can be examined. The failure of antisera to normal cells to neutralize these viruses suggests that the host antigens play no part in neutralization or that the antigens of normal host cells are altered when in combination with the virion.

(B) The envelope antigen is the only known antigen of the C-type viruses to which the natural host does not reveal immunologic tolerance. It follows that if the natural host has any immunologic ability to resist C-type virus activity, this resistance would be mediated against the envelope antigen and any non-virion antigen that the virus might be responsible for (to date none are known). The relationship of tumorigenesis to virus envelope antibody is currently unknown. The presence of circulating envelope antibody may enhance tumor growth by blocking antigenic sites, thus interfering with the host's natural cell bound immunity.

On the other hand, by varying immunization schedules, doses, routes of administration, antigen solubility, there are probably effective ways of immunizing hosts with envelope antigens or inactivated whole virus, so as to confer an immunity to their naturally occurring tumors.

This is at present highly speculative; however, the availability of antisera specific for envelope antigen would allow the study of naturally occurring envelope antigen and envelope antibody and their relationship to tumorigenesis. These studies should be accompanied by simultaneous studies of cell bound immunity in the host. If there is a way of preventing spontaneous neoplasia by classical virological methods, this seems like the most logical approach. The availability of envelope antisera would aid our understanding of the virus-host cell relationship and would allow the study of another function of the C-type virus independently from its other functions. Studies of the site of envelope antigen synthesis and its relationship to virus assembly would be important to establish. Whether unassembled envelope antigen is present on the surface of virus-infected cells and its quantitative relationship to whole virus could be studied.

(C) Fusion of cells has already been proven to enhance the expression of DNA viruses in several systems. It is therefore reasonable to assume that C-type virus expression might be enhanced in susceptible hybrids.

(D) It appears that indigenous hamster C-type viruses can consistently be obtained from hamster tumors induced by murine sarcoma viruses. This phenomenon is somewhat unexpected, when one considers that with few exceptions, C-type viruses are not detectable by the conventional screening methods of complement-fixation, electron microscopy, or by direct isolation attempts of various organs and tumors from uninoculated hamsters and hamsters bearing DNA virus-induced tumors. Infectious virus probably exists in these animals but in quantities too small to be detectable by these methods. The reason for the ability to detect infectious HaLV in MSV-induced tumors is not known but is suggestive of "enhancement" of a latent virus. The failure to detect any HaLV activity *in vitro* in hamster embryo fibroblasts infected singly with a defective sarcoma virus suggests that "enhancement" of HaLV may require other conditions not present *in vitro*; but it does grow when put into fibroblasts. One possibility is that HaLV is a passenger from another source such as the hematopoietic tissues. An approach to this question using cell fusion techniques is in progress. It is obvious from these studies that conventional screening methods are too insensitive to make conclusions about the absence of C-type viruses in species when they have not yet been found.

Proposed Course:

(A), (B) and (C): Continuation of studies outlined above.

(D) Finish the rescue studies and the co-carcinogenesis studies.

Honors and Awards:

None.

Publications:

Kelloff, G., Huebner, R.J., Chang, N.H., Lee, Y.K., and Gilden, R.V.: Envelope antigen relationship among three hamster-specific sarcoma viruses and a hamster-specific helper virus. *J. Gen. Virol.* 9: 19-26, 1970.

Kelloff, G., Huebner, R.J., Oroszlan, S., Toni, R., and Gilden, R.V.: Immunological identity of the group-specific antigen of hamster-specific C-type viruses and an indigenous hamster virus. *J. Gen. Virol.* 9: 27-33, 1970.

Kelloff, G., Aaronson, S., and Gilden, R.V.: Inactivation of murine sarcoma and leukemia viruses by ultra-violet irradiation. *Virology* 42: 1133-1135, 1970.

Huebner, R.J., Kelloff, G.J., Sarma, P.S., Lane, W.T., Turner, H.C., Gilden, R.V., Oroszlan, S., Meier, H., Myers, D., and Peters, R.L.: Group-specific antigen expression during embryogenesis of the genome of the C-type RNA tumor virus: Implications for ontogenesis and oncogenesis. *Proc. Nat. Acad. Sci., U.S.A.* 67: 366-376, 1970.

Hampar, B., Kelloff, G., Martos, L., Oroszlan, S., Gilden, R., and Walker, J.L.: Replication of murine and feline RNA containing C-type viruses in human lymphoblastoid cells. *Nature* 228: 857-859, 1970.

Freeman, A.E., Price, P.J., Bryan, R.J., Gordon, R.T., Gilden, R.V., Kelloff, G.J., and Huebner, R.J.: Transformation of rat and hamster embryo cells by extracts of city smog. Proc. Nat. Acad. Sci., U.S.A. 68: 445-449, 1971.

Kelloff, G., Huebner, R.J., Chang, N.H., Oroszlan, S., and Gilden, R.V.: Lack of immunologic relationship between murine sarcoma viruses adapted to growth in hamster cells and hamster-specific C-type viruses. Nature 229: 155-156, 1971.

Huebner, R.J., Sarma, P.S., Kelloff, G.J., Gilden, R.V., Meier, H., Myers, D.D., and Peters, R.L.: Immunological tolerance to RNA tumor virus genome expressions: Significance of tolerance and prenatal expressions in embryogenesis. Proc. N.Y. Acad. Sci., in press.

Hatanaka, M., Kelloff, G.J., and Gilden, R.V.: Induction of sugar uptake by hamster pseudotype sarcoma viruses. Virology, in press.

Oroszlan, S., Foreman, C., Kelloff, G., and Gilden, R.V.: The group-specific antigen and other structural proteins of hamster and mouse C-type viruses. Virology, in press.

Serial No. NCI-4925
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Ecology and Epidemiology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of C-type RNA Tumor Viruses

Previous Serial Number: NCI-4913

Principal Investigator: Dr. Padman S. Sarma

Other Investigators: Dr. Robert J. Huebner
Dr. Paul Arnstein
Mr. Horace C. Turner

Cooperating Units: Inside NIH:
Dr. Adi Gazdar, VLLB, NCI

Outside NIH:
Dr. M. Gardner, USC School of Medicine, Los Angeles, Calif.
Dr. J. E. Officer, USC School of Medicine, Los Angeles, Calif.
Dr. R. Gilden, Flow Laboratories, Inc., Rockville, Md.
Dr. L. Vernon, Microbiological Associates, Inc. Bethesda, Md.
Dr. R. Luginbuhl, University of Connecticut, Storrs, Conn.
Dr. B. R. Burmester, USDA Regional Poultry Research Laboratory, East Lansing, Mich.
Dr. David Allen, Massachusetts General Hospital, Boston, Mass.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Serial No. NCI-4925

Project Description

Objectives:

This project is concerned with the development and use of sensitive *in vitro* assay systems for studying the prevalence and behavior of the naturally-occurring feline, avian, rat, and other animal tumor viruses utilizing the methodology developed in large part in this laboratory, such as the rescue and detection of defective endogenous tumor virus genomes by trans-species rescue, co-cultivation technique, detection of non-cytopathogenic infectious C-type virus by complement-fixing antigen induction test, etc. These approaches are being intensively applied to various animal tumor systems having covert (switched off) viral genomes in efforts to "turn on" or rescue overt expressions of the viral genomes, the ultimate objective being to apply methods to studies of human cancer.

Methods Employed and Major Findings:

(A) Prevalence of avian C-type viral antigen in chicken embryos.

In our previous studies, evidence was obtained that the presence of an antigen, identical to the avian leukosis group-specific antigen and C-type viral particles in the embryos of apparently normal chickens, represented the vertical transmission of the avian leukosis virus in a non-infectious state.

Our recent studies indicated that the antigen is present in demonstrable levels in a major proportion of various tissues at all stages of embryo development. Our attempts to concentrate these antigens and demonstrate identity with purified avian leukosis gs-a antigen by immunodiffusion tests have been successful. These studies are being continued.

(B) Avian leukosis viral genome demonstrable by a new technique.

A serial tissue culture line, of hamster tumor tissue culture cells derived from a Rous sarcoma virus induced hamster tumor, has been found to contain an avian C-type virus. This virus can be detected by electron microscopy and by the tritiated uridine labelling technique.

This virus failed to replicate *in vitro* in chicken cells of the C/O type, quail embryo fibroblasts and hamster embryo fibroblasts. Upon co-cultivation of these hamster tumor cells with individually prepared cultures of avian leukosis virus gs antigen-containing embryos, the hamster virus acquired the property of an oncogenicity for quails. Certain CF antigen negative embryo cultures failed to provide this helper function. These preliminary studies strongly suggest that the hamster virus is RSVβ'(0) without a helper virus [RAV(0) or RAV(60)] and that the helper virus present, as vertically transmitted avian leukosis viral genome in SPAFAS and the University of Connecticut White Leghorn chickens, provides the viral envelope and the property of infectivity for the hamster derived "defective" RSVβ(0).

(C) Isolation of a non-cytopathogenic rat cell specific C-type virus from MSV(0).

We isolated a non-cytopathogenic C-type virus from virus stocks of MSV(0), originally isolated and described by Dr. R. C. Ting. This non-cytopathogenic virus replicated in NRK rat cells but not in mouse cells. The virus was neutralized by an antiserum prepared against MSV(0). As recently shown by Dr. S. Aaronson, our studies also suggest that the rat cell-specific, non-cytopathogenic, C-type virus is a rat leukemia virus and that MSV(0) is a rat leukemia pseudotype of Moloney murine sarcoma virus.

(D) Detection of feline leukemia viral antigens in the complement-fixation test with antisera to the group-specific antigens of the murine leukemia viruses.

Broad reactive murine leukemia-sarcoma gs antisera, prepared in rats by the induction of murine leukemia sarcoma virus tumors, are useful and quite as sensitive as feline leukemia gs antisera for quantitative assays of feline leukemia viral antigen and infectious virus. The feline leukemia virus was assayed in feline embryo cultures by a CF antigen induction test, similar to the COCAL test. These studies confirm and extend the observations of Geering, Old, and associates, and the recent studies of Shäfer on the immunological cross-reactions between the various mammalian C-type viruses.

In addition to providing a new tool for detecting the feline leukemia virus, the technique of quantitative CF assays of a C-type virus with antibodies against a related, heterologous C-type virus could have similar, important applications in the detection and assay of C-type mammalian viruses for which suitable techniques or reagents are still not available.

(E) Viral interference in the feline leukemia-sarcoma complex.

A viral interference test was developed for the detection and assay of feline leukemia viruses. The test was initially made possible by the discovery that focus-forming viruses with the viral envelope and host range characteristics of feline leukemia virus can be produced by the rescue of defective murine sarcoma virus genome in hamster tumor cells with feline leukemia virus. The viral interference test was based on the observation that the feline leukemia pseudotypes of murine sarcoma viruses as well as feline sarcoma viruses failed to induce morphological transformation of feline embryo cells productively infected with closely related strains of feline leukemia viruses.

Nine feline leukemia pseudotypes of Harvey strain murine sarcoma virus were produced. Preliminary observations suggest the existence of distinct differences in viral envelope antigens of feline leukemia viruses demonstrable in the viral interference tests. Based on differential viral interference patterns, the feline leukemia virus strains and the feline leukemia virus pseudotypes of murine sarcoma virus derived from these strains and three strains of feline sarcoma viruses were provisionally placed in three subgroups of A, B, and C. The viral interference test may thus prove to be useful in the studies of viral envelope antigens of the viruses of the feline leukemia-sarcoma complex.

(F) Studies of feline sarcoma virus (FSV).

Newly isolated strains of FSV, the ST-FSV and the SM-FSV were obtained through the courtesy of Drs. G. Theilen and S. McDonough respectively. The *in vitro* biological characteristics of these two FSV strains were compared with those of the GA strain of FSV.

These viruses were found to replicate and produce foci of cell transformation in feline, canine, porcine, and human embryo fibroblast cultures. The viruses could be readily assayed in feline embryo cultures by the focus assay technique. All the viruses were found to contain 10 to 1000 fold excess of associated non-cytopathogenic feline C-type viruses. Virus titration patterns in feline embryo cultures gave one-hit curves. The addition of virus neutralizing antiserum to feline cultures, 24 hours after infection, did not result in suppression of the growth of the foci.

All three strains of FSV were found to be susceptible to viral interference only by their associated, non-cytopathogenic C-type viruses and by the feline leukemia viruses of the B subgroup. The FSV strains thus fall under subgroup B of the feline leukemia-sarcoma complex.

(G) Sera of veterinarians tested for complement-fixing and virus neutralizing antibodies against feline leukemia virus.

Eleven sera of veterinarians considered to be presumptive positive by the indirect fluorescent antibody test (Dr. J. Riggs) and 10 "control" human sera were screened for complement-fixing and virus neutralizing antibodies against feline leukemia virus. Positive "non-specific" reactions with control cat cells were noted in the complement-fixation tests. The sera did not inhibit or neutralize 100 focus-forming units of the SM strain of feline sarcoma virus.

Significance to Biomedical Research and the Program of the Institute:

Precise and sensitive *in vitro* techniques are much needed for the isolation and characterization of known and as yet undetected C-type RNA tumor viruses of various animal species. Our efforts in this direction have been successful in detecting and uncovering infectious and/or latent C-type viruses of cats, chickens, and rats.

Studies of the expression of C-type viral genome in avian embryos revealed similarities to the known wide distribution of similar viruses in mice and hamsters and indicated that most chickens are infected with a unique "switched off" virus capable of acting as helper for the defective Rous sarcoma virus [RSVβ'(0)] under certain *in vitro* conditions.

Despite the lack of evidence on the overt expression of the naturally-occurring ratleukemia viruses, the finding of a "helper" "rat C-type" virus in virus stocks of rat passaged murine sarcoma virus [MSV(O)] suggested that rats are similar to hamsters in carrying endogenous largely switched off C-type viruses which are "derepressed" or brought to light by the passage of defective murine sarcoma virus in rats.

The procedure of detecting feline leukemia virus with broad-reacting complement-fixing antibodies prepared in rats against the antigenically related murine C-type viruses suggests the use of similar approaches for uncovering as yet undetected C-type viruses of other species including man.

The biological properties and *in vitro* behavior of three strains of feline sarcoma viruses were established to delineate the similarities and differences between these feline virus strains as well as to the known sarcoma viruses of chickens and mice. Except for differences in focus morphology and growth rate in infected feline cultures, the three feline sarcoma virus strains were found to have identical properties and were similar to the avian and murine sarcoma viruses.

The viral envelope antigens of the feline leukemia and sarcoma viruses were characterized by viral interference techniques which enabled a provisional subgrouping of feline C-type viruses by viral interference patterns. A classification of field strains of feline leukemia and sarcoma viruses by antigenic and viral interference patterns may thus serve not only for epizootiological studies of prevalence and distribution of the virus types by geographic location but also may lead to studies of the possible role of type specific viral envelope antigens in the degree of oncogenicity and ability to cross species barriers under natural and experimental conditions.

These efforts provide tools for further studies of the natural occurrence and spread of leukemogenic and/or sarcomagenic viruses in homologous and heterologous host species including man.

Proposed Course:

Recently developed *in vitro* tests such as the complement-fixation test for cat leukemia virus and trans-species rescue techniques will be used to study the natural history of C-type viral genomes in chickens, rats, and cats. Similar limited studies will be undertaken to examine the presence of C-type viral genome in human tumors. The viral envelope antigens of feline leukemia and sarcoma viruses will be further characterized by viral interference and viral neutralization tests.

Honors and Awards:

None.

Publications:

Baron, S., duBuy, H., Buckler, C.E., Johnson, M.B., Park, J., Billiau, A., and Sarma, P.S.: Induction of interferon and viral resistance in animals by polynucleotides. Ann. N. Y. Acad. Sci. 173: 568-581, 1970.

Gardner, M.B., Arnstein, P., Rongey, R.W., Estes, J.D., Sarma, P.S., Rickard, C.G., and Huebner, R.J.: Experimental transmission of feline fibrosarcoma to cats and dogs. Nature 226: 807-809, 1970.

Sarma, P.S., Huebner, R.J., Baskar, J.F., Vernon, L., Gilden, R.V., and Toni, R.: Feline leukemia virus detection *in vitro*. Virology 41: 377-381, 1970.

Allen, D.W., Sarma, P.S., Niall, H.D., and Sauer, R.: Isolation of a second avian leukosis-group-specific antigen (GS-b) from avian myeloblastosis virus. Proc. Nat. Acad. Sci. 67: 837-842, 1970.

Sarma, P.S., Huebner, R.J., Turner, H.C., Gilden, R.V., and Log, T.S.: Feline leukaemia viral antigens and antisera to the group specific antigens of the murine leukaemia viruses. Nature New Biol. 230: 50-52, 1971.

Sarma, P.S., Gilden, R.V., and Huebner, R.J.: Complement-fixation test for feline leukemia and sarcoma viruses. Virology, in press.

Sarma, P.S., and Log, T.: Viral interference in feline leukemia-sarcoma complex. Virology, in press.

Sarma, P.S., Neubauer, R., and Rabstein, L.: Murine sarcoma and leukemia viral infection of mice. Effect of long-term treatment with Poly I·Poly C. Proc. Soc. Exp. Biol. Med., in press.

Sarma, P.S., Gilden, R.V., and Huebner, R.J.: A complement-fixation test for the detection of viral antigens and infective virus of feline leukemia and sarcoma. J. Amer. Vet. Med. Ass., in press.

Serial No. NCI-4926
1. Viral Carcinogenesis Branch,
OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: (A) Alterations in tRNA Species During Oncogenic Viral Infection

(B) Survey of Naturally Occurring Oncogenic Viral Inhibitors

Previous Serial Number: None

Principal Investigators: Dr. F. H. Portugal

Other Investigators: Dr. J. Simonds

Cooperating Units: Inside NIH:

Dr. George J. Todaro, NCI
Dr. Stuart A. Aaronson, NCI
Dr. Robert J. Huebner, NCI
Mr. W. Lane, NCI

Outside NIH:

Dr. J. Spahn, Microbiological Assoc., Inc.
Dr. M. Hatanaka, Flow Laboratories, Inc.

Man Years:

Total:	1.6
Professional:	1.0
Other:	0.6

Project Description

Objectives:

(A) To determine whether alterations in isoaccepting species of tRNA occur in mammalian cells during infection with RNA (MSV or MSV/MLV) or DNA (SV40) containing viruses.

(B) To determine whether natural inhibitors, which are capable of inhibiting viral induced carcinogenesis, can be isolated from cells infected with oncogenic virus.

The basis of this study is the observation that such inhibitors (STAS) can be isolated from the supernatant media of certain infected mouse cell lines (Nature 227: 173, 1970) using silicotungstic acid reagent.

Methods Employed:

(A) Transfer-RNA isolated from each infected cell line was acylated *in vitro* by standard methods and mixed with RNA from non-infected cells charged with the corresponding amino acid isotope. Double-label chromatography profiles were determined by precipitating acylated tRNA from each fraction and collecting on millipore filters.

(B) Cell lines under investigation include $JLSV_5$, $JLSV_6$, $JLSV_9$, and $JLSV_{10}$.

Preparation of inhibitor follows low speed centrifugation to remove cells. Polyethylene glycol is added to precipitate virus, followed by the addition of silicotungstic acid to remove polyethylene glycol; and after dialysis to remove chemicals, the samples are lyophilized.

Three different assay systems are used to measure inhibitor activity--(1) effect of inhibitor on sarcoma and leukemia virus RNA-dependent DNA-polymerase activity *in vitro* (2) effect of inhibitor on virus production using the XC plaque assay *in vitro*, and (3) effect of inhibitor *in vivo* on MSV or FLV induced tumors or leukemias.

Major Findings:

(A) Data is presently being collected and tabulated by computer. A major and significant change for an isoaccepting species of tRNA has been noted for uninfected cell that have undergone treatment with trypsin and have been cultured *in vitro*.

(B) $JLSV_5$ STAS preparations at a final concentration of 2.5 or 5.0 mg/ml inhibited RLV RNA-dependent DNA polymerase activity. This material had no effect on VSW-polymerase activity. Controls consisting of STAS prepared from unincubated EMEM media containing 5% FCS had no effect on either RLV or VSW-polymerase activity.

The same preparations of $JLSV_5$ STAS also caused 80% reduction of plaques in the XC test using MLV compared to studies in the absence of STAS. Interestingly, the control media STAS preparation was without effect.

No activity with any STAS preparation was obtained *in vivo* with either MSV or FLV. This is in contrast to published observations that such preparations are active *in vivo* in concentrations of 1 mg per animal or lower. $JLSV_5$ STAS, although active *in vitro*, produced no reduction in spleen enlargement or reduction in palpable tumors in animals inoculated with FLV or MSV even at dosages of 200 mg per animal.

Significance to Biomedical Research and the Program of the Institute:

(A) The alterations, if any, of mammalian cell metabolism during oncogenic viral infection are poorly understood. In bacteria, alterations in certain isoaccepting species of tRNA during phage infection provides the virus with an important mechanism for regulating cell metabolism. The present study seeks to determine whether viruses infecting mammalian cells contain information to produce a similar regulatory effect. This information would be of value for understanding the mechanism of cell transformation and viral carcinogenesis.

(B) Isolation of natural products capable of inhibiting viral induced carcinogenesis is of importance as a tool for understanding the molecular basis of carcinogenesis and could ultimately prove of value in restricting viral-induced carcinogenesis.

Proposed Course:

(A) Three cell lines infected with an RNA or DNA-containing virus are being studied presently. Attempts are being made to determine which amino acid, if any, is being affected by the infection and whether the changes are similar regardless of the type of virus the cell has been exposed to.

Survey of 10 to 15 amino acids in each of the three cell culture systems (SV40, MSV, or MSV/MLV infected) has been undertaken.

Codon assay of any new species of tRNA that are detected as a result of infection will be undertaken to determine the genetic code response of the new species.

This information may be of value in understanding how the tRNA species, induced by viral infection, carries out its regulatory function.

Determination of whether the new species of tRNA is specified by information contained in the viral genome or is the result of an alteration of cell metabolism, which results from virus infection.

(B) STAS preparations from additional cell lines including $JLSV_6$, $JLSV_9$, and $JLSV_{10}$ are presently being tested by the multiple assay systems. In addition, similar preparations from homogenized cell pellets are in preparation and will be tested.

Samples of $JLSV_5$ STAS and spleen and thymus STAS prepared by the French investigators have been received and are presently being tested in vivo.

Isolation of inhibitor materials from cell culture supernatants using milder methods of isolation is also being studied.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4927
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Ecology & Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Quantitative Microscopic Survey and Ultrastructural Studies of Viruses in Various Human and Animal Tissues

Previous Serial Number: None

Principal Investigator: Dr. Victor H. Zeve

Other Investigators: None

Cooperating Units: Dr. Adeline Hackett, Univ. of California
Dr. H. Meier, Jackson Laboratory

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

(1) Intensive search for RNA viruses for 25 human fetuses between the ages of 4-6 weeks.

(2) All major organs and tissues will be examined. The strain of human cell lines for RNA virus derived from various types of cancer cell lines indicating 1.16 uridine peak are of prime interest. In addition we are presently comparing the Mason monkey virus (Mel 3) to other known C-type, B-type, and syncytial forming viruses.

(3) To clarify and define the ultrastructure of A-, B-, and C-type RNA viruses and syncytial viruses positively for identification purposes.

Methods Employed:

Presently we are subjecting Mel 3 virus to several fixation techniques for EM in order to determine if virus fixation technique can alter ultrastructural findings. Other known viruses are being treated in an identical manner to serve as controls. Selected representative viruses in thin section will be subjected to reduced electron beam intensity and accelerating voltages to determine the extent of beam damage to the specimen. Image intensification and enhancement by electron storage will be utilized to record images on photographic plates.

Major Findings:

During the short period of this tudy, several interesting ultrastructural changes have been observed as a result of varying fixation techniques. Over fixation in chrome osmium has altered the appearance of the outer coat of monkey foamy virus to the extent that the spiked structure can be obliterated.

Human cell lines indicate a 1.16 uridine peak have been positive by EM for herpesvirus but no C-type RNA virus. Hence, the validity of isotope tagging is being further investigated.

Significance to Biomedical Research and the Program of the Institute:

Extensive expenditure of money has been made for the screening, by EM, of human tumors for RNA virus. Further reports indicate the presence of such agents by EM and isotope tagging. Identification as to what is virus and what type of viruses are present is of great importance to the Program at this point. A critical method to determine and positively identify viruses by EM must be resolved.

Proposed Course:

Continued studies will be carried out to positively identify viruses by the methods outlined in this report.

Honors and Awards:

None

Publications:

Todaro, G. J., Zeve, V., and Aaronson, S. A.: Virus in cell culture derived from human tumour patients. Nature 226: 1047-1048, 1970.

Gerwin, B. I., Todaro, G. J., Zeve, V., Scolnick, E. M., and Aaronson, S. A.: Separation of RNA-dependent DNA polymerase activity from the murine leukemia virion. Nature 228: 435-438, 1970.

Serial No. NCI-4928
1. Viral Carcinogenesis Branch, OASDVO, Etiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies with Syncytium-forming Viruses.

Previous Serial Number: None

Principal Investigator: Dr. Wade P. Parks

Other Investigators: None

Cooperating Units: Dr. Victor H. Zeve, NCI
Dr. Stuart A. Aaronson, NCI
Dr. George J. Todaro, NCI
Dr. Edward M. Scolnick, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

Syncytium-forming viruses have been isolated from several mammalian species and seem to be ubiquitous in these species. In spite of several biologic features which suggest these viruses may have some importance in naturally occurring cancer, relatively little is known about them. This project was undertaken to further characterize these viruses.

Methods Employed:

Standard tissue culture, virologic, biochemical and electron microscopic methods are employed.

Major Findings:

(A) Biologic Properties: A type 3 simian syncytium-forming "foamy" virus isolated from uninoculated monkey kidney cell cultures has been employed as a model for studies with these viruses. One of the major difficulties in working with foamy viruses has been their relatively poor growth; that is, they grow slowly and do not reach high levels of infectivity. In order to determine conditions for optimal virus production, quantitative assays are required. Previous infectivity assays for these agents required approximately 30 days for completion of an assay. We ha e developed a plaque-forming infectivity assay which requires 7-10 days. Quantitative studies using this system revealed that these viruses have a wide host range and grow equally well in cells from diverse species; for example, both rat and human cells support virus growth to titers of 10^5 plaque-forming units per ml. Further, optimal detection of syncytium formation (the expression of viral infectivity) requires growing cells--non-dividing cells show little evidence of virus expressions, but the latent virus genome can be activated by transferring infected normal-appearing cells to conditions which favor cell growth and division. The requirement for host cell growth appears to be analogous to the requirement noted with known RNA-containing tumor viruses.

Ultrastructural studies comparing the morphogenesis of simian foamy virus with two RNA-containing tumor viruses, murine leukemia virus and murine mammary tumor virus have been carried out in collaboration with Drs. Victor Zeve and George Todaro. These studies have revealed differences in the mode of development and size of these viruses. Foamy virions have fully formed nucleoids at all stages of budding and prominent surface projections after budding. Murine leukemia viruses (C-type) form their nucleoid as they bud and have a double inner membrane not noted in foamy viruses. Murine mammary tumor virus (B-type) shows several similarities with the foamy viruses in its pattern of development but has a larger nucleoid, and its surface projections are seen best by negative staining rather than by thin sectioning.

Electron microscopic studies done by Dr. Zeve included collaborative studies with Drs. Todaro, Aaronson, and Scolnick to monitor reverse transcriptase positive sucrose gradient fractions for viral particles. These studies revealed the presence of polymerase activity in the Rauscher viral nucleoid region of sucrose density gradients. These studies suggested that the viral polymerase occupied an internal site within the virion.

Electron microscopic monitoring of murine tumor material for C-type particles from the Jackson Laboratories, Bar Harbor, Maine, was also performed by Dr. Zeve.

(B) Biochemical Studies: We have shown that simian foamy virus incorporated uridine and not thymidine suggesting that the nucleic acid type is RNA. The virus is ether-sensitive and has a buoyant density in sucrose of 1.16-1.18 gm/cc. These data support electron microscopic studies that the virus contains membrane-like material acquired during budding, which surrounds a ribonucleic acid core. Although the nucleic acid type appears to be RNA, foamy virus replication was inhibited by relatively low levels (1-2 μg/ml) of actinomycin D. Since this compound inhibits DNA-dependent RNA polymerase, these studies suggested that a continuing host cell DNA function was required or that the virus replication involved a DNA intermediate.

Since the only viruses possessing these characteristics also contained an RNA-dependent DNA polymerase, foamy viruses were assayed for polymerase activity in collaboration with Drs. Scolnick and Aaronson. Concentrated virus preparations (>10^6 PFU/ml) were found to contain both an endogenous and polymer-stimulated DNA polymerase activity. The polymerase activity cosedimented with the virus in sucrose gradients and had divalent cation requirements similar to those of the Rauscher polymerase. Foamy prototype virus strains type 2 and 3 and foamy type 3 grown in rat, monkey, and human cells all contained DNA polymerase activity. Monkey kidney cells infected with foamy virus also contained a higher polymerase activity than control cells.

We have subsequently examined syncytial viruses from several sources and they syncytium-forming viruses from cats, cattle, and hamsters also contain DNA polymerase activity. The strikingly common biologic and morphologic properties of these virions suggests they compromise a previously unrecognized animal virus group.

(C) Antigenic Studies: Plaque-forming infectivity assays have been employed for virus neutralization studies. The plaque assay procedure enhances the sensitivity of antibody detection at least ten-fold compared to usual end-point infectivity titrations. Employing monkey and cat sera, which react with simian and feline foamy viruses as well as antibody produced in heterologous animals, the feline syncytial virus can be antigenically differentiated from the known simian foamy viruses. Similarly, sera which neutralizes the cat virus does not neutralize any of the simian foamy virus serotypes tested. The bovine and hamster viruses are not neutralized by sera with antibodies against either the simian or cat viruses. These data suggest that syncytial viruses from different species possess distinct envelope antigens.

Significance to Biomedical Research and to the Program of the Institute:

Syncytium-forming viruses from different species constitute a new animal virus group with several interesting properties which suggest these viruses may have a role in natural oncogenesis. These properties include the presence of a virion associated RNA-dependent DNA polymerase and sensitivity to actinomycin D, features common to the known RNA-containing tumor viruses.

Proposed Course:

(1) Further characterization of the virus-cell interaction with syncytium-forming viruses from cats, cattle, and hamsters with particular emphasis on studies to determine if they possess transforming properties.

(2) To characterize the viral ribonucleic acid size and strandedness of the simian foamy viruses.

(3) To identify other syncytium-forming virus antigens.

Honors and Awards:

None

Publications:

Parks, W.P., Scolnick, E. M., Todaro, G. J., and Aaronson, S. A.: RNA-dependent DNA polymerase in primate syncytium-forming ("foamy") viruses. Nature 229: 258-260, 1971.

Scolnick, E. M., Aaronson, S. A., Todaro, G. J., and Parks, W. P.: RNA-dependent DNA polymerase activity in mammalian cells. Nature 229: 318-321, 1971.

Parks, W. P., Aaronson, S. A., Todaro, G. J., and Scolnick, E. M.: Characterization of syncytium-forming virus. Bact. Proc. (IN PRESS).

Serial No. NCI-4928

Todaro, G. J., Aaronson, S. A., Scolnick, E. M., and Parks, W. P.: RNA dependent DNA polymerase in virus and in cells. *Second Lepetit Colloquium.* North Holland Publishing Company, Amsterdam. (IN PRESS)

Serial No. NCI-4929
1. Viral Carcinogenesis Branch,
OASDVO, Etiology
2. Solid Tumor-Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Herpesviruses: Immunologic and Biologic Studies in Relation to Human Tumors.

Previous Serial Number: 4923

Principal Investigator: Dr. Berge Hampar

Other Investigators: None

Cooperating Units: Dr. L. M. Martos, Flow Laboratories, Inc.
Dr. K. Miyamoto, Flow Laboratories, Inc.
Dr. K. C. Hsu, Columbia University

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(1) To characterize the antigens associated with Herpesviruses.

(2) To determine the specificity of human antibodies to Herpesviruses.

(3) To elucidate the mechanism of Herpesvirus latency.

Methods Employed:

Standard tissue culture and virologic methods are employed.

Major Findings:

(A) Structural antigens of Herpesvirus hominus (H. hominus): Identification by immunoferritin:

Early (7 day) and late (7 week) rabbit 7S and 19S antibodies to H. hominus were studied by immunoferritin to determine their specificity for virion antigens. These four antibody fractions were shown previously to differ in their neutralizing properties for isologous and heterlogous virus strains.

Prior to viral morphogenesis, antigens reactive with early 7S and 19S antibodies were found in both the nucleus and cytoplasm, while antigens reactive with late 7S and 19S antibodies were restricted to the nucleus. Viral antigens synthesized in the cytoplasm appear to enter the nucleus through the nuclear pores.

Viral core antigens reacted only with early 7S antibodies. Viral capsid antigens reacted with both early 19S and late 7S antibodies. Blocking experiments, however, suggested that the early 19S and late 7S antibodies reacted with different capsid antigens.

Virus envelopment occurred at the nuclear and tubular cytoplasmic membranes. The initial stages of envelopment were characterized by the appearance of "early" membrane-associated antigens which reacted with late 19S and to a lesser extent with early 7S antibodies. As envelopment proceeded, the membrane area in proximity to the virus capsid thickened so as to resemble the virus envelope, and this thickened area no longer reacted with the late 19S or early 7S antibodies. It appears that the membrane-associated antigens reactive with the late 19S and early 7S antibodies become fixed at a subsurface site in the virus envelope.

Enveloped virus particles reacted with early 19S and late 7S antibodies. Blocking experiments, however, suggested that the early 19S and late 7S antibodies reacted with different envelope antigens.

The significance of these findings may be summarized as follows: (1) The antigenic heterogeneity of H. hominus structural antigens noted by acrylamide gel electrophoresis has now been confirmed serologically and localized to specific virion sites. Of the seven major H. hominus structural antigens identified by electrophoresis, the immunoferritin technique with rabbit 7S and 19S antibodies can identify at least four and probably six antigens. (2) Since rabbits can undergo chronic infection with H. hominus, the demonstrated heterogeneity in the specificity of rabbit antibodies to H. hominus produced at different times after infection may help explain the heterogeneity noted in the properties of human antibodies at different ages following natural infection with this virus. (3) We can now identify and localize those antigens which differentiate H. hominus variants. These antigens, which react with late 19S antibodies, are membrane-associated during viral morphogenesis and become fixed in the virus envelope at a subsurface site. This finding agrees with those reported by others using acrylamide gel electrophoresis.

(4) We now have serologic evidence to support the proposal, based on biochemical evidence, that H. hominus strains should be classified into one serologic group with variants being designated as subtypes. The criteria for this conclusion is that envelope surface antigens (reactive with late 7S and early 19S antibodies) are not significantly different between H. hominus variants to justify the classification of serotypes, whereas antigens which differentiate H. hominus variants are located at subsurface envelope sites (reactive with late 19S antibodies), which suggests that they are not reactive with most commonly employed typing sera. (5) We have demonstrated that H. hominus neutralization may occur at sites distant from the envelope surface. In this case late 19S antibodies, which react with subsurface envelope antigens, can neutralize virus in the presence of complement. (6) The findings by immunoferritin suggest that the enhanced neutralization titers against H. hominus noted with some rabbit antibody fractions in the presence of complement may be related to the antigenic site with which the antibodies react. In each instance where enhancement of neutralization titers by complement has been noted, the antibody fraction can be shown by immunoferritin to react preferentially with subsurface virion sites.

(B) Development of a method for enhancing the yield of HTV positive cells.

Immunoferritin studies with HTV have been hampered by the relatively low percentage of available virus positive cells. A method for enhancing the yield of HTV positive cells has been developed. This involves co-cultivation of HTV positive HR1K Burkitt cells with a continuous line of African green monkey (vero) cells. The vero cells form a monolayer and retain (probably by entrapment) the HR1K cells, which normally grew in suspension. As replication of HR1K cells proceeds, cells are shed into the medium. These unattached cells show percentages of HTV varying from 30% to 70% depending on the time of harvest. The HR1K cells which remain attached to the monolayer (approximately 50%) show low percentage of HTV, and no HTV production occurs in vero cells. The co-cultivated cultures can be serially passaged at weekly intervals and do not require reinitiation. This method should prove useful for obtaining high percentages of HTV positive cells for electron microscopic studies.

(C) Specificity of human antibodies for Herpes-type virus (HTV).

The 7S (γG) and 19S (γM) fractions of human heterophile positive infectious mononucleosis (IM) sera and "normal" human HTV positive sera were conjugated with ferritin and tested against HTV infected cells. Reactivity of the IM sera was localized in the 19S fraction, while reactivity of the "normal" human sera was localized in the 7S fraction. One IM serum (out of 7) showed additional activity in the 7S fraction, suggesting seroconversion prior to collection of the serum.

The anti-HTV activity of the IM and "normal" sera was restricted to capsid antigens, and no reactivity by immunoferritin was observed with core antigens or with enveloped particles. Similar capsid activity has been observed with rabbit anti-HSV sera prepared against capsid particles.

These findings suggest that heterophile positive IM is associated with a primary immunogenic exposure to HTV (or a serologically related virus). This supports the contention that HTV may be the etiologic agent of heterophile positive IM. However, neither our findings nor those reported by others prove that HTV and the virus of IM are identical and not merely related serologically.

Significance to Biomedical Research and the Program of the Institute:

The potential role of Herpesviruses in human or animal tumors requires a more detailed knowledge of the immunology of this virus group. The only plausible way these viruses could be involved etiologically with neoplasia is if the virus can be shown to persist in cells in a latent or masked form. Once virus replication occurs, cell death invariably ensues. Consequently, serologic studies which merely measure virion directed antibodies (e.g., by neutralization) do not afford required information for relating Herpesviruses to neoplasia.

Before one can determine whether antibodies are present to nonvirion antigens (antigens which one would expect if the virus were present in a latent or masked form), we must identify those antigens associated with the virion. Herpesviruses are complex and contain many structural antigens. The absence of neutralizing activity does not prove, a priori, that a serum lacks virion antibodies, since internal structural antigens may not be accessible to the antibody. At the very least, one should include complement in any neutralizing test for the detection of Herpesvirus structural antigens. Further, one cannot assume that absorption of a serum with virions will remove antibodies to all structural antigens.

Our findings by immunoferritin and neutralization indicate that we can identify the major structural antigens of H. hominus. With these reagents in hand, we are in a better position to identify possible nonstructural antigens. However, it is now necessary to develop more simplified techniques for identifying various virus associated antigens, since the immunoferritin technique does not lend itself to large scale screening studies.

One other area where our studies may prove beneficial concerns the question of when a virus which is ubiquitous in a population may be active in a disease process (other than neoplasia). Our approach to this problem envisions changes in the profile of antibodies to various virus associated antigens in sera from "normals" and from individuals with disease associated with virus.

Proposed Course:

Studies as outlined above will be continued and more refined techniques will be sought for detecting Herpesviruses in a latent or masked state.

Honors and Awards:

None

Publications:

Hampar, B., Gerber, P., Hsu, K. C., Martos, L. M., Walter, J. L., Siguenza, R. F., and Wells, G. A.: Immunoferritin and immunofluorescent studies with Epstein-Barr virus (EBV) and herpes simplex virus (HSV) by use of human sera and hyperimmune rabbit sera. J. Nat. Cancer Inst. 45: 75-85, 1970.

Hampar, B. Kelloff, G. J., Martos, L. M., Oroszlan, S., Gilden, R. V., and Walker, J. L.: Replication of murine and feline RNA-containing C-type viruses in human lymphoblastoid cells. Nature 228: 857-859, 1970.

Hampar, B., Miyamoto, K., and Martos, L. M.: Serologic classification of herpes simplex viruses. J. Immunol. 106: 580-582, 1971.

Miyamoto, K., Morgan, C., Hsu, K. C., and Hampar, B.: Differentiation by immunoferritin of herpes simplex virion antigens with the use of rabbit 7S and 19S antibodies from early (7 day) and late (7 week) immune sera. J. Nat. Cancer Inst. (IN PRESS)

Serial No. NCI-4930
1. Viral Carcinogenesis Branch
OASDVO, Etiology
2. Field Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biology of C-type RNA Viruses in Naturally Occurring Neoplasias

Previous Serial Number: NCI-4920

Principal Investigator: Dr. Paul Arnstein

Other Investigators: Dr. Robert J. Huebner
Dr. Padman S. Sarma
Mr. H. C. Turner
Mr. John Estes

Cooperating Units: Dr. M. Gardner, USC School of Medicine, Los Angeles, Calif.
Dr. J. E. Officer, USC School of Medicine
Mr. R. Rongey, USC School of Medicine
Dr. E. H. Lennette, Calif. State Dept. of Public Health, Berkeley, Calif.
Dr. J. L. Riggs, Calif. State Dept. of Public Health
Dr. A. Hackett, Naval Biomedical Research Laboratory, Oakland, Calif.
Dr. W. Nelson-Rees, Naval Biomedical Research Lab.
Dr. L. Hayflick, Stanford University, Stanford, Calif.
Dr. E. Stanbridge, Stanford University

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

Determine the prevalence and establish the biological characteristics of C-type agents in feline tumors and feline fetuses collected through NIH-NCI contracts in the San Francisco Bay area.

Develop and standardize reliable *in vitro* tests for the detection of C-type tumor virus and antiviral antibodies in animal and human specimens.

Determine the prevalence of sarcoma and/or lymphoma virus antibodies in normal and tumor-bearing animals and close human contacts.

Methods Employed:

Establishment of feline tissue culture lines for virus assay. Through the cooperation of local veterinary practitioners, feline and canine fetuses at different gestational periods were obtained from unwanted pregnancies and monolayer cultures were prepared.

Feline fetuses often fail to yield culture cells which can be successfully grown beyond 3-4 *in vitro* passages: of 28 attempts to establish cell lines from 16 fetuses (including whole fetus, brain, liver, spleen, pancreas, ovary, salivary gland, kidney, lung, thymus) only 8 lines were obtained--a salivary gland, two brains, an ovary, a testis, a lung, and a whole fetus.

Examination of feline fetuses for C-type virus particles. Feline fetal organs (mostly liver) were directly examined for C-type particles: 6 of 20 fetuses were positive (1 in thymus, 5 in liver). To date, no C-type particles were seen in normal fetal cells in serial culture, but resistance to FSV transformation has been observed. Several other "normal" embryos reacted in complement-fixation tests with dog antisera containing feline leukemia group-specific antibodies.

Susceptibility of various cells to feline sarcoma virus (FSV). Normal cat, dog, baboon, chimpanzee cells, and other cells derived from feline embryos in this laboratory or from the cooperating NBL laboratories were tested for susceptibility to FSV transformation as soon as uniform monolayers were produced. Virus stock of the GA strain of FSV has now been produced from *in vitro* transformed beagle embryo cells. Several feline cultures, including a fetal salivary gland culture, have been found nonsusceptible to transformation by this virus. Tests are underway to determine if a pre-exisiting C-virus or viral antigen may be responsible.

Use of beagle cultures for feline sarcoma virus assay. Beagle (dog) embryo cells furnished by Flow Laboratories gave the most promising reproducibility in virus assays, but, at passage 30, the cells were difficult to propagate and the foci induced were difficult to recognize. A program of rapid subconfluent serial passage was initiated (similar to the 3T3 system of Todaro). The resulting line termed NBE3RP (Normal Beagle Embryo 3 Rapid Passage) now in 68th passage, is susceptible to FSV producing countable foci at virus dilutions as high as 10^{-4} with both the GA and the ST fibrosarcoma viruses.

In vivo tumorigenesis tests. Progressive fibrosarcomas induced in dogs and cats by the GA strain of FSV have been harvested, cultured, and tested for virus at several passage levels. Some of the cultured feline and canine tumor cells retain a "transformed" appearance in culture, continue to produce C-type virus detectable in the EM and the virus released remains sarcomagenic for at least 14 passages in vitro, inducing fibrosarcomas in recipient newborn kittens and puppies. Other tumors have yielded normal appearing cells, containing feline leukemia immunofluorescent viral antigens but no detectable infectious sarcoma virus.

Through the cooperation with the Southwest Foundation, San Antonio, the susceptibility of fetal baboons to GA-FSV was tested. Preliminary results suggest that fetuses of 100-120 days gestation inoculated in utero with virus or virus containing tumor transplants do not develop sarcoma.

Similarly, GA-FSV materials sarcomagenic for puppies and kittens have been inoculated into newborn rabbits and guinea pigs (random bred). These two species appear to be insusceptible.

Tumors such as feline carcinoma (1), dog osteosarcoma (1), human rhabdomyosarcoma (1), and high passage beagle dog embryo cell (1), were found to produce rapidly growing and sometimes metastatic growth in mice immunosuppressed with antilymphcytic serum (done in collaboration with Stanford University). The tumors were composed of malignant-appearing cells resembling the donor cultures.

Establishment of "tumor" cell lines for virus and rescue studies. Selected tissues from tumored cats were propagated and observed for morphological and growth characteristics and presence of C-type virus particles. Syncytial virus, a ubiquitous cytopathogenic passenger agent, still destroys many cultures by the 4th in vitro passage. The following feline "tumor" cultures have been successfully grown past the "crisis" stage and are available: one squamous cell carcinoma of the tongue, one adenocarcinoma of the breast, and several experimental fibrosarcomas. The experimental fibrosarcomas are C+, the two carcinomas C-. A salivary gland culture from a cat with breast adenocarcinoma is producing C-type virus in vitro (8th and 29th tissue culture passage).

Production of antisera. A pilot experiment is in progress in which parrots and pigeons have been hyperimmunized with several feline C-virus preparations. Three parrots hyperimmunized with electro-focused gs antigen from FeLV have produced group-specific antibodies, detectable by a single precipitin line in gel diffusion tests against feline leukemia gs antigen and other feline leukemia preparations. Preliminary results suggest that one of the parrot sera also reacts with feline leukemia tissue culture viral antigen in CF tests as well.

Several dog and cat sera from animals receiving tumorigenic or immunizing preparations of FSV appear suitable for neutralization or fluorescent antibody tests for feline C-type virus.

Significance to Biomedical Research and the Program of the Institute:

Even though the domestic cat is probably the most "random bred" (as opposed to inbred) of the domesticated animals, it continues to reveal very widespread distribution of C-type virus activity, including overt as well as various levels of covert expressions of the C-type viral genome. It is thus excellently suited as a model for the study of the role of these expressions, both beneficial and detrimental, on the long range survival of the feline species. Lymphoma and fibrosarcoma are well documented naturally-occurring virus-induced diseases in this species. Using the feline viral agents as "handles" for manipulating other tumor virus genomes, the specific viral RNA coding for tumor types of the feline may be incorporated into C-type viral genomes of other species as "pseudotype" moveable oncogenes. These studies may ultimately lead to "rescue" of similar genomes of less "switched on" tumor agents involved in carcinogenesis among species, such as dogs, cattle, and man. The development of a rapid neutralization test demonstrable by reduction of feline sarcoma virus focus-formation or reduction in feline leukemia virus titer by fluorescent antibody test (or both), has useful applications for testing field sera of normal and tumor-bearing animals, to determine exposure to viruses antigenically related to feline leukemia and sarcoma viruses.

Proposed Course:

Continue and amplify studies outlined above.

Honors and Awards: None

Publications:

Gardner, M.B., Arnstein, P., Rongey, R.W., Estes, J.D., Sarma, P., Rickard, C.G., and Huebner, R.J.: Experimental transmission of feline fibrosarcoma to cats and dogs. Nature 226: 807-809, 1970.

Gardner, M.B., Arnstein, P., Johnson, E., Rongey, R.W., Charman, H.P., Turner, H.C., and Huebner, R.J.: Feline sarcoma virus tumor induction in cats and dogs. J. Amer. Vet. Med. Ass., in press.

Hackett, A., Pfiester, A., and Arnstein, P.: Biological properties of a syncytia-forming agent isolated from domestic cats (feline syncytia-forming virus). Proc. Soc. Exp. Biol. Med. 135: 899-904, 1970.

Serial Number NCI/NIAID 71 D
1. Viral Diseases
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of Mouse Leukemia Viruses

Previous Serial Number: Same

Principal Investigators: Dr. Janet W. Hartley, LVD, NIAID
Dr. Wallace P. Rowe, LVD, NIAID
Dr. Robert J. Huebner, VCB, NCI

Other Investigators: Dr. Natalie M. Teich, LVD, NIAID
Dr. Douglas R. Lowy, LVD, NIAID
Dr. Theodore Pincus, LP, NCI

Cooperating Units: Inside NIH:
Dr. Stuart Aaronson, VLLB, NCI

Outside NIH:
Dr. Aaron Freeman, Microbiological Associates, Inc., Bethesda, Md.
Dr. Roger E. Wilsnack, Huntingdon Laboratories, Baltimore, Md.
Dr. Frank Lilly, Albert Einstein College of Medicine, New York, N.Y.

Man Years:

Total:	11.0
Professional:	4.5
Other:	6.5

Project Description

Objectives:

To utilize sensitive virus isolation and quantitation procedures for murine leukemia and sarcoma viruses in studying the natural history of infection with these agents and the biology of their growth in tissue culture.

Methods Employed:

Tissue culture procedures, using plaque and focus assays and complement fixing antigen induction; complement fixation; fluorescent antibody techniques; tumor induction in mice, hamsters and rats.

Major Findings:

Host range studies: Previous studies have shown that naturally-occurring murine leukemia viruses (MLV) initiate infection significantly more efficiently on embryo cells of either NIH Swiss (N-tropic viruses) or BALB/c (B-tropic viruses) mice. Characterization of the sensitivity of embryo cells of 21 other strains of mice to N- and B-tropic MLV's has indicated that all strains tested were similar either to NIH (N-type) or to BALB/c (B-type). Patterns of sensitivity of N-type X B-type F_1 hybrids indicated dominance of resistance to both types of virus, and the sensitivity of cells from individual backcross embryos indicated that a single locus (N-B) is the primary determinant of host range patterns. Further, in collaborative studies with Dr. Lilly, strong evidence was found for the identity of the N-B locus to the FV-1 locus described for sensitivity to Friend leukemia virus.

The N-B locus does not affect the growth of the laboratory passaged MLV's (Moloney, Rauscher, et al.) which propagated equally on embryo cells of all strains as well as F_1 hybrids; however, certain "NB-tropic" variants derived by passage of B-tropic viruses in NIH cells show a markedly decreased ability to infect DBA (N-type) cells and may provide an indicator for delineation of another allele.

The virology of the observed patterns of virus tropism and genetically determined cell susceptibility has been studied intensively, utilizing the quantitative facility provided by the XC plaque test and infectious center procedures based thereon, which permit the study of individual infected cells. Comparison of the characteristics of infection of sensitive and resistant (actually semi-permissive) cells indicated no significant differences in rate or degree of absorption and penetration, and the yields of virus from an infected sensitive or resistant cell are comparable. However, it appears that the resistant cell requires infection by two (or, in some cases, three) virus particles to become productively infected. Multiplicity effects are also seen in sensitive cells, since productive infections early in the latent period are multi-hit events.

Irradiation studies: Studies of the UV inactivation curves of various parameters of MLV infection have shown three classes of sensitivity. Ability of cells to divide, or to be infected with MLV is extremely sensitive. Ability of infected cells to continue virus production is moderately sensitive; while infectivity of free virus, infectivity of virus-covered cells, and ability of infected cells to induce syncytium formation in XC cells is relatively resistant. This dissociation of functions by UV irradiation provides powerful means for studying the infectious process. For example, infection can be halted during the latent period without destroying the infectivity of those cells that have already started virus production. Also, the ability to kill cells without disturbing their ability to affect XC cells forms the basis of the plaque test for MLV infectivity.

Murine sarcoma virus (MSV): The rescue of the defective sarcoma genome from an MSV non-producer mouse cell line was studied quantitatively. It was found that rescue occurred with high efficiency; all cells producing MLV also produced MSV and the latent period of both viruses in individual cells appeared to be identical.

Serology of MLV's: Continuing studies of the serologic character of naturally-occurring MLV's suggest that isolates are divisible into two broad subgroups--"Gross-type" and "AKR-type". The former subgroup comprises Gross Passage A virus and virus strains neutralizable by Gross Passage A antiserum but not by AKR virus antiserum. AKR-type viruses are those efficiently neutralized by AKR antiserum as well as by Gross antiserum.

Virology of the AKR mouse: Of several cell lines established from cultures initiated with small numbers of AKR embryo cells, two lines were obtained which were free of infectious MLV and MLV-related antigens. Various procedures designed to induce MLV production in these cells are being tested with the aim of obtaining a predictable procedure which could allow quantitative studies of the state of the genome and the processes which allow its expression in such cells.

Quantitative studies of the amount of infectious virus in AKR mice of various ages indicate a changing organ distribution of highest titer virus with increasing age, as well as increasing virus titer in the most active sites with age.

MLV isolates from wild mice: In collaboration with Dr. Freeman, two isolates of infectious virus have been made from embryo cell cultures of rural feral mice. Studies are in progress to confirm the source of virus and study the characteristics of these isolates.

Significance to Biomedical Research and the Program of the Institute:

The biology of murine leukemia virus infection is one of the most important and controversial areas of modern biology; it is also one of the most complex and difficult. There is much evidence indicating that the genetic material of these viruses is transmitted as a genetic or epigenetic element in the germ cells, and that the expression of the viral genome is controlled by host genetic factors. The viral genome can become expressed as a result of aging, carcinogens, and possibly from dedifferentiation of cells. The implications of this model for carcinogenesis, differentiation, immunology, and genetics are immense.

Our laboratory has long considered that investment of its effort into developing meaningful techniques for study of MLV would eventually provide the greatest contribution to this problem. Having now developed sensitive procedures for detecting infectivity and antigens of many of the naturally-occurring MLV's, we feel we have accomplished a large part of this objective.

Proposed Course:

With techniques for rapidly isolating, quantitating, and characterizing MLV's now established, a number of studies related to the biology of MLV infection in various strains of mice are in progress to determine the patterns of virus growth and control in relation to spontaneous or irradiation-induced leukemia. Further, the genetic factors affecting expression and distribution of MLV will be studied in hybrids of mice of different N-B type, with different H_2 alleles, and with differing frequencies of spontaneous leukemia.

Efforts will continue in the study of genome expression in the AKR non-producer cells as well as in other mouse cell lines.

Studies on the factors influencing cell sensitivity to MLV infection will continue.

Honors and Awards:

Dr. Janet W. Hartley: Invited presentation, 10th International Cancer Congress, Houston, Texas, May 1970.

Kimble Methodology Award, October 1970.

Publications:

Aaronson, S.A. and Rowe, W.P.: Nonproducer clones of murine sarcoma virus transformed BALB/3T3 cells. Virology 42: 9-19, 1970.

Rowe, W.P., Pugh, W.E., and Hartley, J.W.: Plaque assay techniques for murine leukemia viruses. Virology 42: 1136-1139, 1970.

Pincus, T., Hartley, J.W., and Rowe, W.P.: A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally-occurring viruses. J. Exp. Med., in press.

Pincus, T., Rowe, W.P., and Lilly, F.: A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to Friend murine leukemia virus. J. Exp. Med., in press.

Hartley, J.W., Rowe, W.P., and Wilsnack, R.E.: Detection of murine leukemia virus antigens. Proceedings, Tenth International Cancer Congress, May 1970, Houston, Texas, in press.

BIOHAZARDS CONTROL AND CONTAINMENT SEGMENT

Dr. Alfred Hellman, OASDVO, Etiology Area, Chairman

Mr. William Emmett Barkley, OASDVO, Etiology Area, Vice Chairman

THE DOW CHEMICAL COMPANY (PH 43-65-1045)

Title: Research and Development of Biohazards Containment Facilities

Contractor's Project Director: Mr. Cyril B. Henke

Project Officer (NCI): Mr. W. Emmett Barkley

Objectives: The primary objective is the development of new concepts in the control and containment of potentially hazardous agents to assure personnel safety, protection of experimental materials and environmental control. This objective has encompassed the evaluation of potential hazards associated with oncogenic virus research; study of the state-of-the-art of control and containment technology; development of criteria for the design of virus containment facilities; surveillance and inspection of containment facility construction; conceptual development, design and fabrication of prototype containment laboratories; development of personnel training documents, safety manuals, and containment operational procedures; environmental monitoring of biological secondary barrier systems; and the evaluation of physical and biological parameters which influence the laboratory and animal room environment.

Major Findings: The research facilities which were fabricated under the prototype laboratories development project have undergone extensive investigations. The following conclusions have been demonstrated:

1. Sophisticated containment barrier systems which represent the state-of-the-art in biohazards control and containment technology can be incorporated in transportable containment facilities.

2. The concept of transportability does not compromise the integrity of the laboratory facility.

3. The quality of construction can be better controlled because the fabrication is performed at the construction contractors plant. Experienced craftsmen are available

and labor problems are avoided. In addition, site difficulties do not exist.

4. The fabrication of transportable laboratories can be completed in less than one year. Typical periods for renovation projects of permanent facilities exceed one and one-half years and new construction can rarely be completed within a two year period.

5. The cost of transportable facilities can be consistent with original construction costs for permanent virus containment facilities and renovation costs of existing facilities.

6. Transportable laboratories with self-contained mechanical support systems can be operated by engineering and maintenance personnel with the same proficiency required of personnel to operate permanent facilities.

7. The prototype laboratories can accommodate high hazard research programs without compromising research functions, procedures or the feeling of permenance.

The contractor has developed and evaluated operating procedures which are performed for the protection of personnel from potential hazards associated with experimentation or operations with oncogenic viruses and other microbial agents. It has been demonstrated that adequate environmental control and biological safety can be achieved without impairment to program activity. The two most important characteristics of a successful environmental control and biological safety program were found to be: (1) creating an awareness and appreciation for potential safety problems and (2) implementing practical and consistent operating and safety procedures.

Significance to Biomedical Research and the Program of the Institute: This research and development contract is an integral part of the National Cancer Institute's Special Virus Cancer Program. The continued effectiveness of the Virus research effort of the National Cancer Institute is dependent upon the efficacious operation of the biological primary and secondary barrier systems which have been incorporated in the new virus containment facility. The contractor is responsible for a continuing effort of assessing and reassessing the needs of the National Cancer Institute for new containment techniques, equipment and facilities, consistent with the changing scope of current research. The experience gained from these activities will serve as the basis for making recommendations which may improve the safety of all biomedical research facilities.

The success of the prototype laboratories development project offers a new approach to providing adequate containment facilities to support potentially hazardous virus research programs. The availability of transportable containment facilities will allow the acceleration of virus cancer research by providing contractors adequate facilities to insure personnel safety and validity of results without time delay.

Proposed Course: The current contract year represents the terminal phase of this contract effort. During this period the contractor will continue to provide technical support to the National Cancer Institute for evaluating operating procedures and biological barrier systems used in the National Cancer Institute's virus containment facility.

In addition, the contractor will maintain a group of scientists and engineers, skilled in biohazards control, available to provide technical assistance to SVCP contractors on problems of environmental control, personnel safety and product protection. This consultation service will represent an extension of the responsibilities of the National Cancer Institute's Office of Biohazard and Environmental Control.

Date Contract Initiated: June 25, 1965

Current Annual Level: $125,000

OHIO STATE UNIVERSITY (PH43-65-1001)

Title: Aerosol Transmission of Oncogenic Viruses

Contractor's Project Director: Dr. Richard A. Griesemer

Project Officer (NCI): Dr. Alfred Hellman

Objectives: The purpose of this research program is to elucidate the hazards to man and experimental animals from aerosol exposure to oncogenic viral agents and nucleic acids currently used in laboratory investigations. We wish also to develop an experimental model to study host susceptibility to airborne viral infection and associated malignancies and then to develop methods of controlling the spread of horizontal oncogenic infection. We have already demonstrated that Rauscher leukemia virus and Yaba virus are readily transmitted bv aerosols to mice and monkeys respectively and that the infective aerosol dose approximates the minimal parenteral dose in these species. Conversely, adenovirus type 12 is not readily transmitted by the airborne route to hamsters.

The primary aim of research in progress is to evaluate the potential hazards from oncogenic viral nucleic acids through studies that define the transmissibility, oncogenicity, and host range of viral nucleic acids. A second series of investigations in progress deal with the biohazards associated with feline leukemia and sarcoma viruses.

A. Oncogenic Viral DNA: Parenteral inoculations - Parental inoculation of mice and hamsters with SV40 virus, infectious SV40 DNA, and appropriate control materials have been completed. The hypothesis being tested is that infectious nucleic acids from oncogenic viruses will cross species barriers more readily than infectious whole virus. The results in the susceptible host, the hamster, confirm that infectious SV40 DNA prepared from semipurified virus is oncogenic. The lower incidence following injection of nucleic acid and the absence of tumors in mice may be related to the intervals of time after exposure since not all groups of animals have reached the time after exposure when tumors are expected to appear (250 to 270 days). The sera from all tumor-bearing hamsters have T-antibody as do 10/70 sera from hamsters without tumors 208-264 days after inoculation.

Aerosol exposures - Nucleic acids from oncogenic viruses may be more readily transmitted by the airborne route. To test this hypothesis 36 newborn mice, 83 thymectomized 3-week-old mice, and 33 thymectomized 3-week-old hamsters have been exposed to dynamic aerosols of SV40 virus as baseline controls. Comparable groups of animals will be exposed to aerosols of SV40 nucleic acid when the preparation of a pool of purified viral nucleic acid is completed.

B. Oncogenic Viral RNA: The demonstration by others that RNA tumor viruses contain RNA-dependent DNA polymerase raises the question whether the enzyme is essential in the pathogenesis of viral neoplasms. If this were so, viral nucleic acid without polymerase should not be oncogenic while viral RNA plus the enzyme should cause tumors. To test this hypothesis, we are purifying viral RNA from feline leukemia virus, testing it for polymerase activity, and injecting it into cats with and without the specific polymerase. UV-irradiated FeLV is used as a source of enzyme.

Progress in this experiment has been impeded by the shortage of purified FeLV preparations that show infectious or oncogenic activity. To date only one of the lots tested of Theilen FeLV (obtained from Pfizer) induced leukemia in kittens, two have RNA-dependent DNA polymerase activity and none have been shown to be infectious for tissue culture.

We have extracted several lots of RNA from this virus using the techniques of Veprek, et. al. (Proc. Am. Assoc. Cancer Res., 1970). The material has a 260/280 ratio of 2 to 2.5. Recovery is about 10 to 20% of the theoretical expected yield when it is assumed that all the protein in the sample is viral protein and that 2.0% of the virus is RNA.

C. Feline Sarcoma RNA: Because of the difficulties in obtaining FeLV preparations with oncogenic activity, feline sarcoma virus will be used instead in an attempt to achieve results more rapidly. Three preparations of purified Gardner feline sarcoma virus with particle counts of 10^{10} - 10^{11} per milliliter have been obtained from Electronucleonics Co. and inoculated into six newborn cats to test their oncogenicity. The preparations have polymerase activity. Attempts to infect tissue cultures are in process.

D. Feline Leukemia: The objectives of this segment of the contract are to determine whether feline leukemia virus can be transmitted by the oro-nasal route and to extend the host range studies on feline leukemia by exposing mice to FeLV and cats to murine leukemia virus.

E. Intranasal transmission: The studies on intranasal transmission of feline leukemia have been completed and a manuscript has been prepared for publication. Feline leukemia was successfully transmitted to cats by intranasal exposure. The latent period was longer than that following intraperitoneal inoculation but the difference is probably related to dose.

Not all inoculated kittens, however, developed leukemia. Of 15 kittens inoculated by either route, 10 developed lymphosarcoma, 4 had thymic and lymphoid atrophy, and 1 is still alive 500 days later. We now have evidence for the transition from thymic atrophy to lymphosarcoma and the former is considered to be a previously unrecognized pre-leukemic disease. One of two germfree kittens, inoculated at birth intraperitoneally with a suspension of spleen and lymph node from a cat with thymic atrophy, has developed lymphosarcoma confirmed by biopsy 8 months after inoculation.

Additional studies on excretion of FeLV in saliva, urine, and feces have been delayed by difficulties with the assay system in a collaborating contractor's laboratory. During the next report period we will assay the samples in our laboratory using methods we have standardized for amplification of antigen by tissue culture passage and detection by complement-fixation.

F. Host range: Studies on the susceptibility of cats to Rauscher virus and of mice to FeLV have been completed and a manuscript is in preparation. Rauscher murine leukemia virus did not produce disease in cats within 15 months even when conditions for infection were maximized. Immunodiffusion, electron microscopy, and mouse inoculation methods excluded viral infection without disease. Similarly there was no evidence of infection in mice inoculated with FeLV. The possibility that Gross virus or street viruses might infect cats can not be excluded.

Significance to Biomedical Research and the Program of the Institute: The potential capacity to transmit infectious nucleic acid from tumor viruses will make it feasible to readily cross specie barriers. Removal of the nucleic acid from the protein coat eliminated the requirement for specific viral coat-cell surface interaction. Such a capability will provide the test hosts for evaluation of the oncogenic potential of any virus regardless of its intact host range. Not only will such an *in vivo* and *in vitro* procedure be of great significance in evaluating the possible cause and effect of possible viral induced malignancy, but it will also bring about a greater potential risk in the inadvertent cross-infection of hosts in the environs of the research facility. An understanding of the latter hazard will also be of utmost importance.

Proposed Course of the Project: Aerosol transmission experiments will be continued during the next year. During the next report period, electron microscopic studies of spleen, lymph node and bone marrow cells will be continued to demonstrate virus in the lesions. Additional subpassages in cats will be made with lymphoid tissues from cats that had thymic atrophy to demonstrate infectiousness and to attempt to relate the lesion to the feline leukemia virus. Studies will also continue on the development and evaluation of assay methods for feline leukemia virus and viral antigen. We will further attempt to isolate and transmit *in vivo* and *in vitro* infectious nucleic acid derived from Feline leukemia virus and sarcoma virus.

In order to develop a model system to study the susceptibility of a host to concurrent infection and cross infection, the cat model will be developed. We plan to study this animal as one would humans on chemotherapy, in order to determine his capacity for antibody synthesis, response to chemotherapy, enhancement of malignancy by immunosuppression and look at the significance of hormonal imbalance in the development of malignancy. Both cellular and humoral immunologic responses will be investigated.

Date Contract Initiated: June 22, 1965

Current Annual Level: $137,000

BREAST CANCER VIRUS STUDIES
UNDER THE JOINT SPONSORSHIP OF THE
BREAST CANCER TASK FORCE AND THE SPECIAL VIRUS CANCER PROGRAM

Dr. W. Ray Bryan, Scientific Coordinator in Viral Oncology, Etiology Area

PFIZER INC. (PH43-67-1176)

Title: Electron Microscopy Related to Viral Studies of Human and Animal Breast Cancer

Contractor's Project Director: Dr. J. J. Oleson

Principal Investigator: Dr. H. C. Chopra (until August 14, 1970)
Mr. George Schidlovsky (since August 14, 1970)

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

This is a professional services contract established to provide electron microscopic monitoring and collaborative research in support of virological research on human and animal breast cancer conducted by other investigators and contractors participating in programs of the Breast Cancer Task Force and Special Virus Cancer Program of the NCI.

A. Studies on Human Breast Cancer

The search for viruses in association with human breast cancer involves a close collaboration between this contractor and Georgetown University (Contract PH43-65-53, Dr. W. F. Feller, Principal Investigator). Clinical and pathological studies as well as procurement and processing of specimens used in the virus search are performed by the latter contractor. Preparation of materials for electron microscopy and the EM study of specimens are conducted by this contractor.

B. Studies on Animal Breast Cancer

Electron microscopic studies similar to those on human materials are also made on various animal tumors involved in investigations under other Breast Cancer Task Force projects. Also, EM back-up is provided for virological studies of any viruses isolated, in collaboration with participants in the Special Virus Cancer Program.

Major Findings:

A. Human Studies

A cell line derived from human breast cancer (established by Dr. L. Old, Sloan-Kettering Institute) was found in collaborative studies with Dr. Feller to produce small amounts of virus-like particles resembling the C-type oncogenic viruses of animals. The particles continue to be produced by this culture and efforts are being made to increase their yield sufficiently to permit their characterization and further investigation.

B. Animal Studies

Under the EM guidance provided by this contract, collaborative efforts with other BCTF and SVCP participants (Contracts PH43-63-1146, and NIH 70-2080, respectively) have resulted in:

(1) The isolation and successful propagation in tissue culture of a C-type virus from an adenocarcinoma of the breast of a female Rhesus monkey, as previously reported. Its successful propagation proved that it is definitely a virus. It is not one of the previously known simian viruses, and its morphological resemblance to the known oncogenic RNA viruses of chickens, mice, and cats, suggests that it may be an oncogenic monkey virus. Studies on its further characterization and oncogenicity are being monitored under this contract.

(2) The demonstration of C-type viruses in 6 different transplantable breast cancers of rats being used in other BCTF projects. One of these, the R-35 rat virus, is being further characterized and tested for oncogenic potential in experiments monitored under this contract.

Significance to Biomedical Research and the Program of the Institute:

The collaborative study involving this contract and contract PH43-65-53 is one of two such exploratory projects set up under the Breast Cancer Task Force and Special Virus Cancer Program, to determine whether sufficient evidence can be found for an association of virus with human breast cancer to justify a broader program effort on the viral approach to this human disease. The studies on animal breast cancers not now known to be caused by viruses, are for the purpose of guiding the human studies.

Proposed Course:

This contract will continue to monitor the efforts under other contracts to: (1) increase the yield of C-type particles now being produced in small quantities by an established line of human breast cancer in tissue culture; (2) to establish other human cell lines which may produce more of the C-type particles; (3) to propagate the C-type particles from human cancer milk specimens on various cell substrates in tissue culture; and (4) to determine the potential oncogenicity of the monkey and rat viruses described.

Date Contract Initiated: 6/28/67

Current Annual Level: $91,970

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (PH43-65-53)

Title: Human Breast Cancer Studies

Contractor's Project Director: Dr. William F. Feller

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) Exploratory studies to determine whether viral etiological agents are associated with human breast cancer.

(2) Tissue culture studies in attempts to propagate virus-like particles found in human breast cancer.

Major Findings: (Joint studies in collaboration with Contract PH43-67-1176; see also).

As previously reported, milk specimens procured from a total of 16 women who have had breast cancer (1 breast removed) were fractionated by density gradient centrifugation, and the fractions expected to contain viruses were separated and further concentrated. Examination by thin section electron microscopy revealed virus-like particles definitely resembling the known RNA tumor viruses of animals in 6 (37.5%) of the specimens. A few additional specimens showed less definite but possible virus-like entities.

Similar virus-like particles have now been observed, with low frequency, in a continuous line of cells in tissue culture (the Levine 3 line derived from human breast cancer and supplied by Dr. Old of the Sloan-Kettering Institute), and in 4 of approximately 40 explants of breast cancer tissue that could be maintained *in vitro* for 60 days or more.

Extensive efforts to increase the amount of particles being produced by the Levine 3 line through stimulation with hormones and blastogens are in progress.

The electron microscopic examination of specimens was performed under the collaborative contract (PH43-67-1176).

Significance to Biomedical Research and the Program of the Institute:

One of the major programs of the NCI concerns the study of viruses in relation to the causation of human cancer. The detection by electron microscopy (both under this collaborative project and under another related project--see contract PH43-68-1000) of entities which could represent a virus, in frequent association with human breast cancer, led to the initiation during the year of a more comprehensive program effort on breast cancer under the SVCP.

Proposed Course:

Major emphasis is now being placed on: (1) the procurement of a large number of milk specimens from women with a history of breast cancer, as well as larger volumes of milk from each; (2) attempts to establish new cell lines from breast cancer which may elaborate more of the particles spontaneously; and (3) attempts to propagate the particles from milk in tissue culture substrates.

Date Contract Initiated: 11/19/64

Current Annual Level: $48,872

INSTITUTE FOR MEDICAL RESEARCH (PH43-68-1000)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

To explore human milk and breast cancer tissue in search of a virus that might be etiologically related to the disease.

Major Findings:

(Comment: The support under this contract permits exploratory work on human specimens to be carried on in parallel with studies on the mouse mammary tumor virus (MTV) supported under the grant mechanism.)

Methodology developed in studies with the mouse model system has been applied in the study of human milk specimens. Large virus particles having the size and ultrastructural characteristics of the mouse mammary tumor virus have been observed in 7 (4.5%) of 156 specimens from normal women of the general population in the Philadelphia area. When present, they were found consistently in repeated studies on different samples of the same specimens. Similar particles have also been observed in 6 (60%) of milk specimens from 10 normal women belonging to "high breast cancer" families and whose mothers and/or grandmothers had had breast cancer.

Joint studies with the Tata Memorial Cancer Center of Bombay, India, (supported under PL 480 funds) for the study of milk from women of the inbred Parsi community of Bombay, have shown similar particles to be present in 18 (39%) of 46 milk specimens from normal women of this highly inbred sect.

Significance to Biomedical Research and the Program of the Institute:

This is one of three exploratory projects set up to determine whether sufficient evidence could be found for an association of virus with human breast cancer to justify a broader formal program on the virus approach to the etiology of the human disease. The results of studies under this and the other related contracts (see also PH43-65-53 and PH43-67-1176) led to an expansion of programmed activities on breast cancer during the year.

Proposed Course:

(1) Attempts will be continued to adapt, and improve, methods for the detection of virus developed in the parallel studies on mouse mammary tumor virus.

(2) Special population groups of humans (high-cancer-risk families, and partially inbred populations) will be studied on a larger scale and efforts will be made to procure larger quantities and more samples of milk containing particles so that they can be further characterized.

Date Contract Initiated: 6/28/68

Current Annual Level: $56,788

MASON RESEARCH INSTITUTE (NIH 70-2204)

Title: Hormonal Influences on the Induction of Breast Cancer in Specific Virus Infected Animals

Contractor's Project Director: Dr. Marcus Mason

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) By studies of the hormone profiles of blood and urine of experimental animals during normal physiological cycles and during different regimens of hormone treatment, to determine the hormone dosages, combinations, and sequences to be tested in the virus-hormone co-carcinogenesis studies of objective (2).

(2) To determine whether candidate viruses isolated from animal breast cancers are capable of inducing breast cancers in their natural hosts and/or animals of other species, using appropriate hormone stimulation of the hormone-dependent target tissues (mammary glands).

Major Findings:

The initial studies under this recently established project are restricted to investigations on the Mason-Pfizer monkey virus (M-PMV) isolated from a breast cancer of a Rhesus monkey, and the R-35 rat virus isolated from a transplantable mammary cancer of a Sprague-Dawley rat (the R-35 tumor of the contractor's institute).

Due to a lag period in activating the production of these two viruses under another SVCP contract (NIH 70-2080), the viral inoculation studies (objective 2) have been underway for only a few months and sufficient time has not elapsed for tumor appearance.

Extensive studies on the hormone profiles (objective (1)) are being conducted to guide the hormone treatment of the virus inoculated animals (monkeys, rats, mice and hamsters).

Significance to Biomedical Research and the Program of the Institute:

At the present time, breast cancer is known to be caused by a virus in only one animal species, the mouse. This project, involving biological testing of candidate viruses for oncogenicity, is part of a broader program activity for determining whether viruses are related to breast cancer in animals other than mice. Such animal studies are necessary for developing technology and approaches to the study of the human breast cancer virus problem.

Proposed Course:

The experiments underway will be continued until the planned numbers of experimental animals have been inoculated with virus (50 monkeys, 2000 rats, and several hundred each of mice and hamsters, all inoculated as newborns). Observations for tumor induction will be continued for the lifetimes of the rodents, and for 3-5 years in the case of monkeys.

Date Contract Initiated: 6/9/70

Current Annual Level: $258,600

III. CONTRACT SUMMARIES

SPECIAL VIRUS CANCER PROGRAM
ETIOLOGY AREA, NCI
Fiscal Year 1971

DEVELOPMENTAL RESEARCH PROGRAM SEGMENT

Dr. Robert A. Manaker, Chief, VBB, Etiology Area, Chairman
Dr. Roy F. Kinard, VBB, Etiology Area, Vice Chairman
Dr. Jack Gruber, VBB, Etiology Area, Executive Secretary[1]

AICHI CANCER CENTER (NIH-69-96)

Title: Virus Rescue Studies in Human Leukemia/Lymphoma Cell Lines

Contractor's Project Officer: Dr. Yohei Ito

Project Officers (NCI): Dr. Jack Gruber
Dr. Virginia C. Dunkel

Objectives: (1) To establish cell lines *in vitro* from human neoplasms and examine these for virus or antigens by electron microscopy, immunology and transformation experiments. (2) To supply human embryonic cell cultures and lymphoma-type tumor tissues available in the Far East.

Major Findings: Efforts to establish continuously growing cell lines from human neoplastic tissues were resumed as one of the main lines of study in the second year of the contract. In addition to the cultures from neoplasia of the hematopoetic system, cell cultures from solid tumors such as nasopharyngeal carcinoma (NCP) were also attempted. This was done because of the well established fact of high herpes-type virus (HTV) antibody titer in the sera of patients with the disease. Among 13 NPC specimens cultured, 8 gave rise to monolayer culture, of which half showed morphological alteration. From these cultures, one free-floating cell line was established. The presence of HTV antigen in these cells was demonstrated by direct immunofluorescence test.

To obtain an established cell line which grew in a floating state with less or hopefully no HTV antigen, cultures from hyperplastic tonsils of children were carried out. Of 136 specimens, 12 cell lines were established as free-floating cells. The ratio of cells containing HTV antigen was relatively small but HTV antigen was detected in all the cell lines.

Some 50 strains of cells were maintained in the laboratory and they served as a procurement center for the supply of the cells for research workers in the area. The procurement of human embryonic cultures was also continued into the second year. About 40 human embryos in total were processed for such culture. Human sera of high HTV antibody titer were also supplied to colleagues of the SVCP.

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[1] Replaced Dr. Roy Kinard as Vice Chairman on March 2, 1971.

Seroepidemiological studies using indirect immunofluorescence test have been continued to accumulate more data on the HTV antibody titer of individuals with various neoplastic diseases and normal subjects. However, a hope to reveal a new disease with high HTV antibody titer turned out to be fruitless so far.

Contacts have been strengthened with the institutes of the Asiatic area to provide access to the human tumor material and serum specimens which might be useful to the SVCP.

Significance to Biomedical Research and the Program of the Institute: This project will supply supporting data from Far Eastern sources to supplement information obtained in the U.S. on the association of viruses with specific neoplastic diseases.

Proposed Course: In general, studies initiated previously will be continued. A new aspect of the work scope is the introduction of biochemical techniques to search for the presence of RNA-dependent DNA polymerase among the approximately 90 cell lines established from human neoplastic tissue during the past two years. Furthermore, fresh human cell materials from leukemic and lymphoma patients at the Aichi Cancer Center Hospital will be tested for polymerase activity. Such studies would provide new data on neoplastic cells from patients of oriental origin. Additionally, plans are to study the *in vitro* effect of various chemical carcinogens on established lymphoblastoid cell lines, and to initiate new investigations on other human neoplasms where virus activity is suspected.

Date Contract Initiated: May 2, 1969.

Current Annual Level: $40,000

BAYLOR COLLEGE OF MEDICINE (PH43-68-678)

Title: Studies on Viruses as Related to Cancer with Emphasis on Leukemia

Contractor's Project Director: Dr. Joseph Melnick

Project Officers (NCI): Dr. Jack Gruber
Dr. Roy Kinard

Objectives: (1) To isolate, propagate and identify viral agents to provide evidence of association with human neoplasia and (2) to continue to hold and observe primates inoculated with suspected cancer viruses or cancer tissues.

Major Findings:

A. Viral etiology of leukemia and mononucleosis.

Propagation of selected lymphoblastoid (Ly) cell lines from patients with leukemia or mononucleosis and normal individuals is continuing, for use in immunological and biophysical studies.

Comparative chromosomal analysis of 16 lymphoblastoid cell lines cultivated up to 61 months revealed no specific association between the presence of EB virus and any of the chromosomal anomalies observed. Regardless of the source of cells or the presence of EB virus, cells with marker chromosomes or trisomies appear to have a selective advantage of growth in vitro, as documented by the increase in frequency of clones with these anomalies with progressive passage.

Studies were continued to search for antibody to possible tumor antigens in acute leukemia of childhood. Peripheral blood leukocytes are being reacted with serial autologous serum samples by indirect membrane immunofluorescence (IMIF). In autologous tests, cells from 20 of 25 children in remission have yielded negative results and 5 questionable. Cells obtained from 6 children during relapse were also tested for autoantibody. The IMIF assay has been uniformly negative for all sera and autologous relapse phase cells tested so far.

A 78 A1 cell line of rat embryo fibroblasts that had been transformed by the murine sarcoma-leukemia virus (MSV-MLV) complex is being used as a model for characterization of RNA species obtained from various human Ly cell lines. The 68S RNA of MSV-MLV was found to dissociate after heating or dimethylsulfoxide treatment into 37S, 18S and 4S subunits, differing in base composition and buoyant densities in cesium sulfate. A double-stranded DNA with a sedimentation coefficient of 7S density was isolated from highly purified MSV-MLV. This DNA was complementary to the 18S subunit, but not to the 37S or 4S subunits of the viral RNA. Work is being initiated to follow infectious virus synthesis and macromolecular synthesis during de novo infection and transformation of cells by MSV-MLV.

B. Comparative studies on herpesviruses.

Studies continue on the distribution of complement-fixing (CF) antibody to EB virus-induced S antigen in groups with various disease entities and in normal individuals. The results are compared with those obtained in the immunofluorescence (IF) test with fixed EB3 cell preparations. Sera from 21 patients with sarcoidosis, kindly supplied by Dr. Phillip Glade, revealed both IF and S antibody to EB virus, a result similar to that reported earlier for patients with post-nasal carcinoma. A longitudinal study of the development of IF and S antibody starting with newborn children is being conducted. The results to date indicate that: (1) IF antibody to EB virus appears within several months after maternal antibody has disappeared, indicating an early infection with this agent. (2) There is a lag of several months between the appearance of IF and S antibodies. (3) Infection with the other three herpesviruses (herpes simplex, cytomegalovirus and zoster virus) occurs much later than infection with EB virus.

Work continues to purify and characterize the soluble CF antigens extracted from two EB virus-positve (EB3 and P3J) and from one EB virus-negative (NC37) Ly cell lines. Ly cell line RPMI 8226, free of both EB virus and soluble CF antigen, is used as negative control. Studies on the non-serum-requiring complement-fixation (NSR-FCF) by EB virus-positive Ly cells indicate that the reaction may be measuring an EB virus directed antigen-antibody. Attempts are being made to identify antibody to EB virus in extracts of Ly cells.

The biochemical and biophysical properties of the DNA of HSV type 1 and type 2, infectious bovine rhinotracheitis (IBR) and Pseudorabies (Pr) viruses were compared. DNA-DNA hybridization was employed to investigate genetic relatedness between these four members of the herpesvirus group. Saturation and competition hybridizations demonstrated greater than 90% homology between three strains of HSV-1 and less than 5% homology between HSV-1, Pr virus and IBR virus. Saturation hybridization indicated at least 90% homology between two strains of HSV-2, but no homology between HSV-2 Pr virus and IBR virus. Preliminary experiments indicate that there is less than 50% homology between the DNAs of type 1 and type 2 HSV.

Further attempts to obtain additional BUDR-induced ts mutants of HSV have resulted in the isolation of 50 potential mutants. The 22 original ts mutants of HSV have been tentatively assigned to 8 complementation groups. Groups so far contain from 1-4 mutants. Before detailed characterization of representative members of complementation groups is undertaken, repeat tests are planned using both the 22 original mutants and the newly isolated mutants mentioned above.

The protein and glycoprotein synthesis by a HSV temperature-sensitive mutant (ts 343) and the wild-type virus has continued to be compared by polyacrylamide gel electrophoresis. Evidence was obtained that mutant ts 343 does not synthesize glycoprotein C5, a major envelope protein, at the nonpermissive temperature (40°). Studies are in progress on the glycoprotein synthesis of additional HSV ts mutants. Preliminary findings indicate that the glycoprotein profiles of mutants belonging to separate complementation groups may be significantly different.

C. Role of herpes virus type 2 in cervical carcinoma.

Seroepidemiologic studies of women with invasive cervical cancer and controls from Uganda and Israel failed to reveal the same strength of association between antibodies to herpesvirus type 2 and malignancy, as previously observed in Houston, Atlanta, Baltimore and Brussels. Studies in different populations are continuing. Recently, 118 cervical cancer patients and 83 controls from Muslim and Christian women in Yugoslavia were studied. No difference in type 2 herpes antibodies was found between Muslim cases and controls, but the occurrence of antibodies among Christian women was twice that of control women. In New Zealand, the occurrence of antibodies was only slightly higher in the patients than in the control women. In the USA most of the studies that yielded a high incidence of antibodies were carried out among women of the lower socioeconomic group. Thus, in populations of different composition and different life styles, differences are found in the association between type 2 herpesvirus and cervical carcinoma.

Type 1 strains are susceptible to cytoside arabinoside and IUDR (iododeoxyuridine), inhibitors of replication of DNA-containing viruses. However, type 2 strains are resistant to these DNA antagonists. This is apparently due to the low levels of thymidine kinase which are characteristic of type 2 strains in contrast to the high levels of this DNA-synthesizing enzyme found with type 1 strains.

D. Immunofluorescent cell surface antigens in human tumors.

Cell lines have been established from human melanoma and sarcoma tissue. Attempts are being made to separate tumor cells from the normal cells by (1) density gradient centrifugation, (2) by cloning out tumor cells, and (3) by injecting the mixture into immunosuppressed mice. With one of the two melanoma cell lines tested, the patient's serum reacted in the membrane fluorescence test. Sera from other melanoma patients reacted positively for antigen in the cytoplasm of the tumor cells. Lymphosarcoma cells reacted in the membrane fluorescence test with autologous sera, and cross reactions were seen with sera from other lymphosarcoma patients.

Significance to Biomedical Research and to the Program of the Institute: This contract provides a progressive comprehensive research program to determine the significance of viruses in human neoplasia. Techniques used are tissue culture, immunology, electron microscopy, primate inoculation, cytogenetics and nucleic acid homology.

Proposed Course: Investigations will continue to detect the presence of nucleic acid characteristic of the RNA tumor viruses in human tumor cells. Immunological studies with antigens associated with EB virus will be continued. Further serologic data will be acquired to aid in determining the relationship between the venereal herpes hominis type 2 virus and cervical carcinoma.

Date Contract Initiated: June 27, 1963

Current Annual Level: $800,000

BIONETICS RESEARCH LABORATORIES, INC. (NIH-71-2025)

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Directors: Dr. John Landon
Dr. David Valerio
Dr. Robert Ting

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber
Dr. Robert Gallo

Objectives: (1) Evaluation of long-term oncogenic effects of human and animal viral inocula in primates of various species, especially newborn macaques; (2) maintenance of monkey breeding colonies and laboratories necessary for inoculation, care and monitoring of monkeys; and (3) biochemical studies of transfer RNA under conditions of neoplastic transformation and studies on the significance of RNA-dependent DNA polymerase in human leukemic tissues.

Major Findings: This contractor continues to produce over 300 excellent newborn monkeys per year. This is made possible by diligent attention to reproductive physiological states of female and male breeders. Semen evaluation, artifical insemination, vaginal cytology and ovulatory drugs are used or tried as needed.

Inoculated and control infants are hand-fed and kept in modified germ-free isolators. They are removed from isolators at about 8 weeks of age and placed in filtered air cages for months or years of observation. The holding area now contains approximately 1200 animals up to 5 years old. Approximately 300 are culled every year at a rate of about 25 per month. This is necessary to make room for young animals inoculated with new or improved virus preparations.

During the past year macaques were inoculated at birth or in utero with the Mason-Pfizer monkey mammary virus, Epstein-Barr virus, Herpesvirus saimiri, and Marek's disease virus. EB virus was given with immunostimulation and immunosuppression (ALS, prednisone, imuran). Australia antigen was given to newborn African green monkeys.

The breeding and holding colonies were surveyed for antibody to EBV. All breeders were positive and their offspring contain maternal antibody for several months. Colony-born offspring that have lost maternal antibody and are sero-negative will be surveyed periodically for conversion to the EB positive state.

An RNA-dependent DNA polymerase similar to that associated with RNA tumor viruses was detected in human leukemic cells but not in normal cells stimulated by phytohemagglutinin. The enzyme was isolated, purified and concentrated 200-fold, making possible its further characterization and study in relation to the leukemic process in man.

Significance to Biomedical Research and to the Program of the Institute: Inasmuch as tests for the biological activity of candidate human viruses will not be tested in the human species, it is imperative that another system be developed for these determinations and, subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. Further study of altered transfer RNA and polymerase enzymes would determine their significance in neoplastic change and provide a basis for selection of therapeutic agents.

Proposed Course: Continuation with increased emphasis on monitoring and intensive care of inoculated animals to determine if active infection occurs, effects of infection, and degree of immunosuppression when used. Further studies of human neoplasms at a molecular level will continue.

Date Contract Initiated: February 12, 1962.

Current Annual Level: $2,011,046

CHICAGO PARK DISTRICT, LINCOLN PARK ZOO (PH43-65-1017).

Title: Marmoset Breeding Colony

Contractor's Project Director: Dr. Lester E. Fisher

Project Officer (NCI): Dr. Roy Kinard

Objectives: To provide marmosets in a quantity and quality sufficient for the needs of the research on tumor viruses conducted under Contract NIH-71-2032 with Rush-Presbyterian-St. Luke's Hospital.

Major Findings: The marmoset breeding colony is regularly providing newborn animals at the rate of 100 per year.

Significance to Biomedical Research and the Program of the Institute: This contract is part of a program utilizing lower primates for testing selected laboratory specimens for oncogenic activity. The marmoset, a small, inexpensive primate is under investigation to determine its value for cancer virus research.

Proposed Course: The project will be continued to insure the availability of experimental animals of quality.

Date Contract Initiated: June 28, 1965.

Current Annual Level: $21,550

HEALTH RESEARCH, INC. (PH43-63-593)

Title: Biological and Electron Microscopic Studies of Viruses from Leukemia Cells, Tissues and Plasma.

Contractor's Project Directors: Dr. Edwin Mirand
Dr. Julius Horoszewicz

Project Officer (NCI): Dr. Jack Gruber
Dr. Roy F. Kinard

Objectives: To study the EB virus associated with cells cultured from Burkitt's tumor.

Major Findings:

A. Animal studies with EB virus.

Hematologic studies were done on a total of 34 EBV-injected and uninjected adult male Long-Evans rats; approximately one half of the animals were subjected to partial hepatectomy within 24 hours prior to virus injection intravenously; three different virus preparations were used. There is no indication that injection of EBV induces a sustained leukocytosis, or that hepatectomy has any effect per se in the response of the rat to EBV injection.

Cultures of the buffy coat from heart blood have been made from all experimental and control rats. It appears that the buffy coats from EBV-injected rats grow somewhat faster and better than those from uninjected rats; there is no significant difference between cultures from hepatectomized and intact animals. There appear to be cytological changes in some of the cells of the buffy coats from virus-injected rats suggestive of host response to the virus and/or the foreign human 64-10 cells used as virus-carriers in the preparations injected; further study is necessary before any conclusions can be drawn.

B. Quantitative infectivity assay for EBV.

The practical range of EB virus titration was increased and extends now from 2.5×10^2 to 1×10^6 infectious units per ml. This was accomplished by introducing total counts of immunofluorescent cells in place of estimates based on percentages only. The previously reported procedures to concentrate and store EB virus were found to preserve quantitatively the infectious properties of viral inocula.

During time studies on localization of viral antigens and virus formation in EBV infected cells, Early Antigen (EA) first appeared 6 hours post-infection and was localized in the cell nucleus. Nuclear Capsid Antigen (CA) could be detected 9 to 10 hours post-infection. Soluble Antigen"Beta" (SAB) accumulates in the cytoplasm at 16 hours. Assembly of viral nucleocapsids begins at 14 to 16 hours and "coating" reaction specific for new membrane antigen (MA) becomes positive at 20 to 24 hours post-infection.

Significance to Biomedical Research and the Program of the Institute:
The development of a more sensitive quantitative assay is important for further characterization of the EB virus and for assessing the quality of infectious virus preparations. It is hoped that purified non-virion antigens may be useful in the control of infection by EBV.

Proposed Course: This contract will terminate on June 30, 1971.

Date Contract Initiated: April 18, 1963.

Current Annual Level: $95,050.

KAROLINSKA INSTITUTE (NIH-69-2005)

Title: Studies on the Significance of Herpes-Type virus in the Etiology of Some Human Cancers.

Contractor's Project Director: Dr. George Klein

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Jack Gruber

Objectives: To study the surface membrane antigens of lymphoblastoid cells infected with Epstein-Barr virus (EBV).

Major Findings: Studies were continued on EBV-associated reactions in relation to Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's disease, in comparison with other malignancies. Antigenic studies on carrier cultures revealed that (1) VCA-positive cells are always membrane antigen positive, (2) VCA-early antigen positive cells exist with a variable frequency in different cell lines, (3) VCA-early antigen positive cells can be either positive or negative for membrane antigen, and (4) membrane antigen positive-early antigen and VCA negative cells occur with variable frequency, in different EBV-carrier cell lines. The number of membrane positive-VCA negative cells can be increased by small doses of mitomycin, actinomycin or X-irradiation. In lines with a high frequency of membrane antigen-positive cells, the antigen expression was maximal during the G1 phase of the cell cycle and lymphoblastoid cell lines derived from Burkitt's lymphoma and NPC show a similar spectrum of membrane antigen reactivity.

Antigenic studies on virus infected cells showed that (1) no VCA is made in the presence of DNA inhibitors; (2) membrane antigen is an early product of the viral genome; (3) early antigen starts to appear at the same time or slightly later than membrane antigen; and (4) VCA when observed appeared 3-4 days after infection and at a low level (1-2%).

In two Burkitt's lymphoma patients studied in detail, anti-early antigen antibody titer increased and anti-membrane antigen antibody titer decreased in parallel with tumor recurrence. Anti-VCA titers, and titers against five unrelated viruses remained unchanged in both patients. In patients treated with BCG there is a temporary increase in anti-membrane antigen titers. With repeated treatment there was often a tendency toward diminishing efficiency. Local radio-therapy to tumors in Burkitt's lymphoma or NPC patients results in an increase in anti-membrane antigen titer. The same increase in titer does not take place in patients with "control" tumors, that is, anti-VCA positive donors with malignant tumors other than Burkitt's lymphoma or NPC.

Developmental and exploratory work is being carried out with (1) cell-mediated immunity; (2) osteogenic and other sarcomas; (3) characterization of the hybrid human-mouse cell line, and (4) fusion of A9 cells with other mouse tumor lines and studies on the release of C-type particles.

Significance to Biomedical Research and the Program of the Institute: The membrane antigens present on tumor cells are important since new membrane antigens have been demonstrated on all virally induced experimental tumors investigated whether the interaction with the causative virus was productive or non-productive. This project was undertaken to provide data on the cell membrane antigens and reactive antibodies in specimens from patients with different diseases with which EBV has been associated. A compilation of all data relating to the virus will help to ascertain whether the virus has etiologic significance.

Proposed Course: Current studies will be continued. Attention will be directed to the serological patterns in Burkitt's tumor and other lymphoreticular malignancies, the relationship of membrane antigens to the viral envelope, antibody spectra in relation to disease category and tumor status, levels of membrane reactive antibody in relation to tumor progression, regression, and recurrence, and to the possible existence of fine antigenic differences between the membrane antigen complexes on Burkitt's tumor and nasopharyngeal carcinoma derived cells. Further efforts will be made to measure cell mediated immunity in Burkitt's lymphoma. Collaborative investigations on patient immune responses to sarcoma antigens will be continued.

Date Contract Initiated: April 9, 1969

Current Annual Level: $73,600

Merck and Company, Inc. (NIH)-71-2059)

Title: Study of Viruses in Human and Animal Neoplasia.

Contractor's Project Director: Dr. Maurice R. Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Jack Gruber

Objectives: To perform investigations designed to develop vaccines or other agents effective for the prophylaxis and therapy of human neoplasia of suspected viral etiology.

Major Findings: This is a new contract.

Significance to Biomedical Research and the Program of the Institute: Current data support the concept that a virus or viruses are the essential element in most animal tumors studied and that viruses are probably the necessary etiological component in human neoplasia, though expression may be greatly influenced and modified by host and environmental factors. If viruses are the essential element in human cancer, then prophylaxis by vaccines to prevent or minimize infection should provide a rational approach to cancer prevention. This could be accomplished by utilization of live or killed virus vaccines or possibly by vaccines of purified virion subunits.

Vaccines would obviously provide their greatest benefit in preventing infection with oncogenic viruses transmitted horizontally after birth. However, even the possible vertical transmission of hypothetical neoplastic agents does not rule out a potential benefit from vaccines. Nononcogenic viruses may function as essential cofactors in expression of neoplasia, and immunity against such secondary agents might prevent expression of the neoplastic state. Additionally, antibody or cellular immunity may be enhanced by vaccination with homologous virus in virus-dependent cancer. Obviously this research investigation is of fundamental importance to the goals of SVCP and can make unique contributions to the total program.

Proposed Course: The investigators will devote initial efforts to developing methods for propagation, purification, concentration and specific quantitation of candidate viruses suspected or shown to cause cancer in man. At the present time, investigations will be focused upon herpes-type (DNA) viruses and "B" and "C" type (RNA) particles. Parallel studies to evolve live attenuated and killed virus vaccines in appropriate animal model systems will be conducted. Particular attention will be given to developing and applying optimal methods for viral attenuation, viral inactivation, viral quantitation, vaccine safety assessment, and vaccine potency assay.

Date Contract Initiated: March 1, 1971

Current Annual Level: $1,523,600

MT. SINAI SCHOOL OF MEDICINE (NIH-69-2078)

Title: Studies of EBV and Lymphoproliferative Diseases.

Contractor's Project Directors: Dr. Kurt Hirschhorn
Dr. Phillip Glade

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Jack Gruber

Objectives: To investigate the relationship of herpes-type virus (EBV) to different diseases with a lymphoproliferative phase in order to determine whether EBV is a specific etiologic agent with multiple modes of biologic experession or an adventitious entity which emerges under the stimulus of the lymphoproliferation resulting from the underlying disorder.

Major Findings: Vaccinia immunization appears to increase the potential of circulating lymphoid cells for long term proliferation in vitro and the lymphoproliferative effects consequent to vaccination may stimulate HLV antibody synthesis in vivo. In addition, high antibody levels to HLV in sarcoidosis may reflect a specific event in this disorder, since similar alterations were not found for poliovirus antibody. High antibody titers to HLV may correlate with the degree of impairment of cell-mediated immunologic responses in patients with sarcoidosis. Similar increased levels of anti-HLV reactivity were not found in patients with granulomatous colitis.

Sarcoidosis does not support the thesis of Dennis Burkitt that chronic R.E.S. stimulation influences the host response to HLV with an increased incidence of lymphoreticular neoplasms.

Studies in patients with sarcoidosis and lepromatous and tuberculoid forms of leprosy indicate that cell-mediated immunity has a direct bearing on the degree of the humoral antibody response of the host to infection with the herpes-like virus (HLV Epstein-Barr). While anti-HLV antibody was found in all of these patients, the high anti-HLV antibody titers usually occurred in individuals who had significantly impaired delayed-type hypersensitivity as monitored by skin testing. These findings indicate that it may no longer be tenable to assign an etiologic role to HLV based upon high antibody titers in patients without an assessment of their cell-mediated immune responses. It is quite possible that high anti-HLV antibody results secondarily from the impairment of cellular immunity associated with disease rather than primary overwhelming infection with the HLV.

Studies utilizing the newly developed lymphoid cell line migration technique suggest that high circulating levels of anti-HLV antibody have effects on lymphoid cells which may alter their functional capacities to react. Further studies demonstrated a significant alteration in the P3J clone of Jijoye Burkitt lymphoma cells in terms of their ability to respond to their own inhibitory products. These studies may provide further information about the persistence of HLV in lymphoid cells and perhaps a specific lymphoid defect in Burkitt's lymphoma.

Significance to Biomedical Research and the Program of the Institute: The association of EBV with all cases of sarcoidosis studied and the high antibody levels detected suggest a specific relationship of the virus to this disease and raises the possibility that the agent may be a necessary co-factor in several disease conditions including Burkitt's lymphoma and nasopharyngeal carcinoma in addition to a probably direct etiological relationship to infectious mononucleosis.

Proposed Course: Investigations on EBV relationships in lymphoproliferative conditions will continue. Studies of the *in vivo* consequences of impaired delayed hypersensitivity for anti-HLV serum reactivity will be expanded. *In vitro* studies to elaborate specific deficits in the immunocompetence of Jijoye Burkitt cells as well as the mechanisms favoring latency and persistence of HLV in lymphoid cells will be undertaken.

Date Contract Initiated: June 17, 1969.

Current Annual Level: $83,182.

OHIO STATE UNIVERSITY (NIH-69-2233)

Title: Application of Radioiodine Labeled Antibody Technique to Studies of Virus-Induced Tumors.

Contractor's Project Director: Dr. David S. Yohn

Project Officers (NCI): Dr. Jack Gruber
Dr. Virginia C. Dunkel

Objectives: To apply the paired radioiodine labeled antibody technique (PRILAT) to the detection of virus induced tumor antigens in human tumor cells.

Major Findings: Various forms of the paired radioiodine labeled antibody technique (PRILAT) have been evaluated for sensitivity and quantitative applicability to viral oncology using Adenovirus-12, Rous sarcoma virus and feline leukemia virus model systems. Direct, indirect and inhibition (blocking) procedures have been found applicable. Direct PRILAT yields highly accurate quantitative estimates of the antigen content of test samples or individual cells. Indirect and inhibition of direct PRILAT are highly sensitive methods to detect low levels of antibody. Antibodies have been detected to Ad-12 T-antigen and to avian leukosis group specific antigens(s) in sera which are negative by other serologic procedures.

Significance to Biomedical Research and the Program of the Institute: The project is designed to develop and apply the paired radioiodine labeled antibody method as a very sensitive tool for the detection of specific reactions between humoral antibodies and antigens on the surfaces of tumor cells. Because the method permits discrimination of specific and non-specific binding of serum globulins, it has considerable promise in the study of human neoplasms.

Proposed Course: The main thrust of this project for the next year will be to apply the various PRILAT procedures to the search for tumor specific antigens in human tumors and to detect antibodies in human sera. Liaison is being established with the Departments of Surgery and of Hematology of the Ohio State University Hospital for this purpose. Collaboration is also being established with the M. D. Anderson Hospital in Houston which will also be providing sera and cell lines from human tumor conditions for testing.

Date Contract Initiated: June 27, 1969.

Current Annual Level: $80,000

THE PENNSYLVANIA STATE UNIVERSITY (NIH-70-2024)

Title: Studies on the Oncogenic Potential of Defective Human Viruses.

Contractor's Project Director: Dr. Fred Rapp

Project Officers (NCI): Dr. Virginia Dunkel
Dr. Jack Gruber

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: The effect of herpes simplex virus on mammalian chromosomes was further analyzed. It was determined that the virus generally induces the abnormalities observed in human embryonic lung cells while the cell is in the G2 phase. As it was previously shown that DNA synthesis is not required, the results suggest that a virus coded enzyme (perhaps a DNAse) is responsible for the aberrations observed. Additional characterization of herpes simplex virus mutants revealed that variants picked for failure to replicate in 39° C in rabbit kidney cells grew equally well at 33° C in both rabbit and hamster cells. Detectable infectious virus was not produced at the higher temperature in either cell type but cytopathic effects were observed. The extent of these effects at the high temperature appear to be related to multiplicity of infection.

A model has been established for herpes simplex virus latency in human embryonic lung cells. Treatment of these cells with ara-C in the presence of herpes simplex virus type 2 was followed by a period of 7 days when no infectious virus could be recovered after removal of the drug. After that time, small amounts of virus appeared and approximately 1 to 4,000 cells were induced to release virus. The model allows experimental manipulation for a period of 7 days for the further suppression or activation of this virus and the study of the event underlying latency.

Hamster cells that were morphologically "transformed" following treatment with ultraviolet irradiated herpes simplex virus were tested for oncogenic properties. The cells induced tumors when inoculated into newborn, but not adult, inbred hamsters. These tumors have been transplanted to young adult hamsters and are also being grown in cell culture. Preliminary examination of the cells by electron microscopy has failed to reveal evidence of particles resembling viruses. These cells are now being characterized for herpes simplex virus markers and for virus-specific nucleic acids. Cell lines established from the "transformed" colonies developing after treatment with DMBA treated virus have also been further characterized. Karyotype analysis revealed that one cell line is lacking an acrocentric chromosome. Comparative studies of this line with another line that appears to have a normal complement of 44 chromosomes have been carried out. In neither cells has herpes simplex virus antigen been detected using rabbit antiserum prepared against the virus nor has infectious virus been recovered. However, herpes simplex virus type 1 replicates well in both cell lines, but herpes simplex virus type 2 (the virus to which these cells were originally exposed) replicates very poorly, if at all, in the cell line with 43 chromosomes; this virus does replicate in the cells with 44 chromosomes. Both cell lines have been inoculated into newborn hamsters to determine whether they have oncogenic potential.

Significance to Biomedical Research and the Program of the Institute: The neoplastic response to viral infection is a slow process in contrast to the rapid response to viruses associated with common diseases in man. The abortive infection of cells with some common viruses which are rendered defective by specific treatment raises the question whether such cells in time may undergo neoplastic change. This project is designed to investigate this possibility.

Proposed Course: The studies initiated will be continued and expanded. Major emphasis will concern the hamster cells which have possibly been transformed *in vitro* and the tumors that were induced by these cells. Hopefully, additional tumor-bearing hamsters will be obtained and additional animals will be inoculated in an effort to expand the system. Transplantation experiments using inactivated HSV followed by live virus will be carried out to determine whether this will render the animals resistant to transplantation of these cells in an effort to further link the HSV genome with properties of the transformed cells. Attempts will be made to isolate herpes-like DNA from these cells; as DNA from the virion has a different density than mammalian cell DNA, it is hoped to isolate the DNA by density gradient centrifugation.

Date Contract Initiated: October 27, 1969.

Current Annual Level: $291,850.

CHARLES PFIZER AND COMPANY, INC., (NIH-70-2080)

Title: Facility for Tumor Virus Research and Related Service Activities

Contractor's Project Director: Dr. J. J. Oleson

Project Officers (NCI): Dr. Jack Gruber
Dr. W. Ray Bryan
Dr. Roy F. Kinard

Objectives: Provide research and services related to the isolation, production, purification, assay, and control of tumor viruses, including electron microscopy, tissue culture, and immunology applied to the study of animal and potentially oncogenic human viruses.

Major Findings: Large quantities of a variety of viruses were produced for studies by different investigators of the SVCP. Epstein-Barr virus, feline leukemia virus, Mason-Pfizer monkey mammary virus, and Rauscher virus grown in human cells were the principal viruses produced. Considerable amounts of EBV were produced and assayed for infectivity. Some progress has been made in the study of early and late antigen synthesis following infection with EBV. Large amounts of lymphoblastoid cells originating from different donors were regularly supplied for investigators on human cell membrane antigens, cell enzymes, and soluble EB virus-mediated antigens.

Biological and serological studies of the feline leukemia virus and the Rauscher virus grown in human cells were carried out.

Specific rat antisera to the GS-3 antigen were prepared. These antisera would react with cell antigens from Hodgkin's disease, Burkitt's lymphoma, and chronic myelogenous leukemia but not with normal cells, lymphoblastic lymphoma or MPMV infected monkey cells.

The monkey mammary virus was produced for specific antisera production in monkeys, determination of the antigenic composition of the virus, studies on virus replication, and its biochemical characteristics including polymerase assays. The assay for infectivity of this virus has been increased 10 to 100-fold in sensitivity using immunofluorescence. The virus would not cross-react with the group specific antigen of the Moloney sarcoma virus, the Rauscher leukemia virus, the Rous sarcoma virus or Marek's disease virus. Excellent yields of this virus can be obtained from suspension cultures using infected human lymphoblastoid cells (NC37). The NC37 grown virus has the antigens of the monkey cell grown material and has excellent polymerase activity. Virus is more conveniently produced in this cell line.

A summary of the patient survey case studies completed to date has been made. Two human cell cultures, subjected to arginine depletion or activation with ultraviolet light, X-irradiation and treatment with Mitomycin C, become moderately reactive with the serum of the original patient and with EBV positive control serum. This genome is not detectable prior to activation. Cell cycle and cell synchrony studies have been initiated with the objective of facilitating the activation of hidden genomes.

Immunoferritin tests using electron microscopy have been developed for several viruses and are in use to determine the localization of cell and viral antigens and in cross-reaction studies.

Electron microscope services were provided for outside investigators and supported the contractor's activities.

Significance to Biomedical Research and the Program of the Institute: Since its inception, this contract has provided support to individual investigators thereby making possible research which could not otherwise have been undertaken. The research conducted in the contractor's laboratories has largely been directed to improving the quality of material support to different research activities.

Proposed Course: The production of virus and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961.

Current Annual Level: $1,340,987

ST. JOSEPH'S HOSPITAL (NIH-69-2074)

Title: Study of Human Sarcomas and Possible Viral Etiology.

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officers (NCI): Dr. Albert J. Dalton
Dr. Roy F. Kinard

Objectives: To find and supply fresh human sarcomas or other tumors which contain EM evidence of virus particles, and to attempt to establish cell cultures from some of these tumors.

Major Findings: From the Tampa Bay area hospitals, 234 sarcomas were collected for study. Survey of the tumor tissue and screening for viral particles by electron microscopy reveals the presence of C type particles and filamentous, reticulated material (provirus?) in one case of malignant lymphoma, reticulum cell type, and the presence of virus particles in one case of liposarcoma. Cultures of the tumors are started now routinely and are being screened for particles by EM.

Study of surface characteristics of sarcoma cells is initiated by freeze etching technique.

Sera for the Serum Bank was collected on a total of 226 patients, 39 from patients with breast cancer.

Significance to Biomedical Research and the Program of the Institute: This is one of the projects for the primary search for viruses in human tumors. As many fresh tumors as possible must be examined by EM if viral etiology is to be determined in reasonable time.

Proposed Course: Continue with collection and screening of sarcomas, and of tumor cultures by EM. Summarize the experience gained in ultrastructure of the tumors studies for publication. Utilize two new techniques now available to gather further information: Freeze etching and fluorescent antibody techniques.

Date Contract Initiated: June 24, 1969.

Current Annual Level: $70,000

RUSH-PRESBYTERIAN-ST. LUKE'S HOSPITAL (NIH-71-2032)

Title: Studies of Tumor Viruses in Small Primates.

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber

Objectives: To induce viral neoplasia in small primate species, especially marmosets, and to continue to develop the marmoset as a laboratory animal for viral oncology.

Major Findings:

I. Marmosets.

Marmosets were inoculated with a variety of oncogenic or suspected oncogenic viruses, including: Feline sarcoma and leukemia, Rous sarcoma virus, Marek's and turkey virus, Herpesvirus saimiri, and human leukemia and sarcoma material.

II. Feline Sarcoma Virus.

Tumor transplantation: Transplantation is currently in the 10th passage; these tumors develop in animals 15-18 months old. The first tumor passage was of recipient karyogram; later passages were of both recipient and donor karyograms.

Focus Assay: A direct focus assay for FSV in marmoset and feline cells in culture was established. A manuscript describing the method is in preparation.

Biochemical Studies: A paper titled "RNA of tumors of subhuman primates induced by feline fibrosarcoma virus" has been submitted to J. Virol. Three classes of RNA (15S, 38S and 60-70S) were recovered from FSV-induced marmoset tumor tissue and from marmoset tumor cells grown in culture.

III. Feline Leukemia Virus.

Gs antigen: Semi-purified antigen of FLV was produced and FSV gs-antigen is being produced by the same technique. Guinea pig antisera against FLV-gs antigen with titers of 160 to 320, and reacting in gel diffusion with 2 specific lines, was produced. The possibility that the second line represents interspecies gs-antigen is under evaluation.

Immunoelectroosmophoresis (IEOP), was used for screening for gs-antigen and gs-antibodies and was found to be more sensitive than other gel diffusion methods, and particularly good for screening anti-complementary animal sera for gs-antibodies. A short technical note on a comparison between the sensitivity and specificity of CF, IEOP, standard- and micro-gel diffusion methods is in preparation.

IV. Herpesvirus saimiri (HVS): A pathogenesis study was completed. Two papers are in preparation describing the characteristics of in vitro infection and disease in vivo. Disease in WL marmosets was prolonged with diffuse cell infiltrations and peripheral leukocytosis of up to 200,000 cells per mm^3.

V. Immunological Studies:

Preliminary data were obtained for induction of delayed hypersensitivity to DNCB in marmosets and for immunosuppression with ALG. Significant depression of lymphocyte counts were obtained with anti-patas and anti-human ALG.

Mixed lymphocyte reactions by microtechnique were used to demonstrate stimulation between lymphocytes of unrelated marmosets and lack of stimulation between lymphocytes of twins.

VI. STAS Inhibitor: A 80-90% inhibition of focus formation in vitro with RSV was observed in all experiments with all materials prepared with silicotungstic acid, including mock inhibitors prepared both by the French group and Dr. Chirigo's group with saline instead of leukemia virus. No inhibition was observed with any materials in in vivo inoculation of quails.

Significance to Biomedical Research and the Program of the Institute: As one of the small primates, the marmoset is an excellent animal for laboratory research. The contractor has developed a breeding colony of these animals and has overcome many of the problems associated with their breeding, care and nutrition. The marmoset has been shown to be susceptible to oncogenesis by several known tumor viruses. Sufficient numbers of newborn animals now are becoming available to expand studies on selected human neoplasms suspected of having a virus etiology.

Proposed Course: Continue as described with emphasis on inoculation of newborn marmosets with human tumor specimens or cell lines and viruses derived from them.

Date Contract Initiated: March 15, 1962.

Current Annual Level: $510,860

UNIVERSITY OF TEXAS, M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (PH43-65-604)

Title: Studies of Relationship of Viruses to Human Neoplasms.

Contractor's Project Director: Dr. Leon Dmochowski

Project Officers (NCI): Dr. Jack Gruber
Dr. Roy F. Kinard

Objective: To institute and pursue a systematic study of selected human patients with neoplastic diseases to detect the presence of viruses or virus-mediated antigens.

Major Findings: Further immunofluorescence studies have extended observations that sera of some patients with osteosarcoma contain antibodies against antigens in cells from osteosarcomas. Antibodies in sera of patients with osteosarcoma apparently detect internal and not cell surface antigens of osteosarcoma cells. Cells of a tissue culture derived from a patient with osteosarcoma gave cytoplasmic fluorescence with 6 of 9 sera from patients with osteosarcoma. Anti-feline leukemia virus serum also gave cytoplasmic fluorescence with cells of this culture.

Soehner-Dmochowski virus (SD-MSV) derived from hamster bone tumor could be adapted to induce bone tumors in mice by passage in mouse embryo cells in tissue culture with no loss of bone tumor-inducing activity in hamsters. Infant New Zealand Black rats are almost 100% susceptible to the SD-MSV. SD-MSV induces three types of tumors in these rats: Soft tissue tumors, osteolytic and osteogenic bone lesions. Comparable tumors were also induced in Fisher, Sprague-Dawley, ACI and (ACI-x NB) F1-hybrid rats by NC rat-adapted SD-MSV.

Electron microscope studies utilizing the ruthenium red staining technique have shown that acid mucopolysaccaride (AMS) plays a role in attachment of type C and type B virus from the cells of various tumors of animals of different species.

The majority of sera from patients with breast cancer reacted in the fixed immunofluorescence (FIF) test to give cytoplasmic and nucleolar fluorescence with cells derived from breast cancer and osteosarcoma.

A good correlation has been demonstrated between mixed hemadsorption (MHA) and fixed immunofluorescence (FIF) tests for detection of antigens of type B and type C particles. The MHA test was found to be 100 times more sensitive than the FIF test.

Sera from some melanoma patients gave nucleolar FIF with their own and/or homologous melanoma cells, but not with a variety of normal human embryonic cells, normal human skin cells, HeLa, KB, or HEp-2 cells. The presence of nucleolar antigen is associated with rapidly growing tumors. The nucleolar FIF reaction may provide a means of detecting progression in tumor growth and help in determining the type of treatment.

The use of the colony inhibition test of Hellstrom showed lymph node cell-mediated immunity and possible blocking serum factors in kittens and cats apparently immunologically tolerant to feline leukemia and sarcoma viruses.

A monolayer culture producing type C virus particles has been established from cells of a plueral effusion of a child with Burkitt's lymphoma (American type). No virus particles were found by electon microscope examination in the cells of the original pleural effusion nor in cells of passage 1. Type C virus particles were observed in cells from passage 10 and budding, immature and mature type C particles have been consistently observed through 20 tissue culture passages. The cell line has been designated ESP-1. Mixed hemadsorption tests with anti HeLa cell serum indicate human origin of the cell line. Preliminary karyotyping also indicates a human culture with 90 to 300 chromosomes.

Significance to Biomedical Research and the Program of the Institute: This well organized program for the systematic study of selected cancer cases was instituted to apply existing and newer methodology to an in-depth study on the association of viruses or viral antigens with human cancers. The project should also expand current observations to determine their significance, if any. The data acquired should permit decisions regarding the effectiveness of the precedures applied, and develop areas for intensive investigation. The difficulty in determining the etiological significance of any virus or tumor-associated antigens with oncogenicity in man requires assimilation of considerable supportive information. This project is designed to meet this need.

Proposed Course: Continuation of a systematic study of human tumor cases with emphasis on osteosarcoma and soft tissue sarcomas, melanoma, and mammary carcinoma.

Date Initiated: March 19, 1965.

Current Annual Level: $620,000

IMMUNOLOGY GROUP

Dr. Paul H. Levine, VLLB, Etiology Area, Chairman
Dr. Herbert J. Rapp, CG, Etiology Area, Vice-Chairman

CHILDREN'S HOSPITAL OF PHILADELPHIA (PH43-66-477)

Title: Interference and Immunofluorescence Studies with Cell Lines Derived from Leukemias and Lymphomas

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: (1) To assess by immunologic, virologic, and epidemiologic means, the relationship of EB virus to Burkitt's lymphoma and infectious mononucleosis; (2) To devise methods for improved propagation of the EB virus.

Major Findings: Immunofluorescence studies on cells from biopsies of Burkitt's lymphoma and nasopharyngeal carcinoma revealed that in only 5 of 79 Burkitt's lymphoma biopsies were cells found which contained EB viral antigens. When biopsy cells were placed in culture, EBV antigen-containing cells appeared within 3 to 7 days in 68% of the preparations representing from 0.1 to 5% of the total cell population.

Exposure of human lymphocytes to EBV derived from the HR1K cell line led to establishment of five continuous lymphoblastoid cell lines. Co-cultivation of Gibbon lymphocytes with x-irradiated EBV positive IM cells resulted in the establishment of lymphoblastoid cell lines. These cells were determined cytogenetically to be of Gibbon origin and contained EBV. Injection of such cells into autochthonous animals failed to produce tumors or any other signs of illness, but the animals developed antibodies to EBV within seven to ten days.

The separation of early antigen from viral capsid antigen necessitated retesting of sera from patients with various EBV-associated non-malignant and malignant diseases. Preliminary findings suggest that the early antigen is more disease-associated than the viral capsid antigen, and that patients with infectious mononucleosis, Hodgkin's disease, chronic lymphocytic leukemia, and Burkitt's lymphoma, were more likely to have antibodies to the early antigen even with low VCA titers. Normals generally did not have antibodies to the early antigen, even with high VCA titers.

Significance to Biomedical Research and the Program of the Institute: Herpes-type viruses have been shown to cause lymphoreticular diseases in chickens, rabbits, and monkeys. This contract has contributed a great deal of knowledge to our understanding of EBV infection and the definition of antigens and antibodies relating to EBV. The studies performed by the contractor support the hypothesis of an etiologic role for EBV in lymphoma.

Proposed Course: The contractor will concentrate on the characterization of the early antigen and the evaluation of its importance in human lymphoma by seroepidemiological studies.

Date Contract Initiated: January 1, 1966

Current Annual Level: $115,150

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NIH 70-2076)

Title: Seroepidemiology Studies of Nasopharyngeal Carcinoma and Burkitt's Lymphoma

Contractor's Project Director: Dr. G. Blaudin de Thé

Project Officer (NCI): Dr. Robert H. Depue

Objectives: To investigate the relationship of EBV to nasopharyngeal carcinoma, Burkitt's lymphoma, and other malignancies.

Major Findings: Several lymphoblastoid cell lines were developed from patients with nasopharyngeal carcinoma. The culture conditions for optimum production of EB virus have been determined, and two selective lines have been sent to Pfizer for large scale-production and standard antigens for the IF test. The radio-labeled antibody test (PRILAT) has been developed in conjunction with immunofluorescence tests for studying of EBV antibodies.

Significance to Biomedical Research and the Program of the Institute: High EBV antibody titers have been associated with nasopharyngeal carcinoma and Burkitt's lymphoma. Since fulfillment of Koch's postulates in humans is unethical, the most direct evidence for etiology can be obtained by combining epidemiological and virological expertise. This contract, developed through collaboration of investigators of international repute, provides a program through which evidence of the etiology of EBV to PNC can be evaluated.

Proposed Course: Case control studies on PNC will be developed. The sera collected will be tested for EBV-related antibodies by PRILAT and immunofluorescence.

Date Contract Initiated: January 1, 1971

Current Annual Level: $267,065

TRW SYSTEMS GROUP (NIH 70-2200)

Title: Viral Antigens and Anti-Viral Antibodies

Contractor's Project Director: Dr. Norman Weliky

Project Officer(s) (NCI): Drs. Vincent W. Hollis and Tibor Borsos

Objectives: To purify, separate, and characterize the antigens in Rauscher leukemia virus and the corresponding anti-viral antibodies.

Major Findings: High capacity immunoadsorbents, prepared by crosslinking mouse serum with ethyl chloroformate or glutaraldehyde, are effective in removing all mouse serum antibodies from antiserum except that to a Beta-lipoprotein. Mouse Beta-lipoprotein isolated with dextran sulfate inhibits the antiserum reaction, but it does not absorb antilipoprotein antibody as an immunoadsorbent after chloroformate crosslinking. The lipoprotein antigen was not found in the supernate from a chloroformate crosslinker mouse serum preparation. The large amounts of crosslinking reagents in the system are likely to block the antigenic determinants.

Significance to Biomedical Research and the Program of the Institute: Antigens crossreacting with murine and feline leukemia virus have been reported in human leukemic cells, and immunofluorescence and two human cell lines by immunodiffusion (GS III). This contract, by purifying and separating all the antigens associated with a known animal leukemia virus (Rauscher leukemia virus) will provide direct evidence to confirm or refute the existence of the crossreacting viral antigens in animal and human tumors.

Proposed Course: Adsorbents will be prepared by coupling serum and Beta-lipoprotein to higher capacity particles by a variety of coupling methods, including aldehyde, bromo, activated carboxyl, and diazonium coupling. Physical adsorption, followed by chemical coupling, will be pursued. Quaternary ammonium Sephadex and other polymers will also be used. Preparations will be made using purified Beta-lipoprotein so that its reactivity may be better determined. Retention of the antigenicity of Beta-lipoprotein with blocked side chain reactive groups will be observed.

Date Contract Initiated: June 15, 1970

Current Annual Level: $124,060

Dr. J. B. Moloney, OASDVO, Etiology Area, Chairman
Dr. Louis R. Sibal, OASDVO, Etiology Area, Executive Secretary

MERCK INSTITUTE FOR THERAPEUTIC RESEARCH (PH43-64-55)

Title: Study of Viruses in Human and Animal Neoplasia

Contractor's Project Director: Dr. Maurice Hilleman

Project Officer (NCI): Dr. James T. Duff

Objectives: During the contract period work was performed in the following general areas: (1) immunoprophylaxis and therapy of cancer with tumor cell antigens, (2) chemoprophylaxis and therapy of cancer with interferon indicers, and (3) reorientation and expansion of the program for development of human cancer virus vaccines.

Major Findings: Efforts to prepare killed intact or disrupted tumor cell vaccines and purified tumor cell antigens which retain protective efficacy against tumor growth were continued. Supportive work, such as the development of additional animal-tumor model systems for the assay of tumor cell vaccines, likewise was continued. Three additional studies concerned with the following aspects of cancer immunology were initiated: (1) the study of ineffective tumor cell vaccines in terms of host response including the influence they may have on the ability of the host to respond to a protective cellular vaccine, (2) the use of poly I:C as a possible means of enhancing host response to tumor cell vaccines, and (3) the investigation of the role of the "embryonic" antigen of SV40 tumor cells in protective vaccines.

Attempts are being made to confirm the work of published procedures by other investigators on (1) SV40 tumor cell ghost vaccines, (2) adenovirus 31 tumor cell fraction vaccines, (3) adenovirus soluble tumor cell antigens, and (4) the role of "embryonic" antigens in tumor immunology. Two attempts to confirm published studies on the protection of adult hamsters after vaccination with SV40 tumor cell ghosts against subsequent challenge yielded negative results.

Emphasis was placed on initiating a new program concerned with tumor virus vaccines.

Significance to Biomedical Research and the Program of the Institute: The studies of neoplasia carried out by this laboratory are aimed at the control of cancer in man by immunization and/or therapeutic means.

Proposed Course: The workscope was incorporated into Contract NIH 71-2059 on March 1, 1971.

Date Contract Initiated: October 1, 1963

Current Contract Level: $165,550

FLOW LABORATORIES, INC. (NIH 70-2015)

Title: Oncogenicity Testing of Potential Vaccines Including both
DNA and RNA Virus Studies

Contractor's Project Director: Dr. Raymond Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The objectives of this contract are to determine if vaccine cell substrates carry information for synthesis of C-type viruses, to determine if vaccine viruses interact with highly susceptible cells or animals to cause transformation or tumor induction and to determine if vaccine viruses interact with known type C viruses or their genomes to produce transcapsidants in which an oncogenic virus genome may be present within the envelope of the vaccine virus or a contaminating C-type virus indigenous to the cell line used for vaccine production.

Major Findings: Sera reactive with the mammalian C-type virus interspecies antigenic determinant (gs-3, interspec) have been prepared in rats by hyperimmunization with homogenates of tumors induced in rats by murine sarcoma virus. Such sera react in gel diffusion tests with fractions from isoelectric focus separations which contain species specific determinants. Since these protein fractions are homogeneous when assayed by polyacrylamide gel electrophoresis, both specific and interspecific determinants are assumed to reside on the same molecule. This was found true of mouse, hamster, and cat viruses. In certain sera, activity was found in both 7 and 19S antibody fractions. This could account for the double precipitin lines occasionally observed with these sera. The cross reactivity observed is in fact identical to the interspec reactivity defined by Schafer based on exchange of reagents. Thus far, in a limited survey, no gel diffusion reactions have been obtained between the interspec antisera and human specimens.

Proposed Course: Contract was combined with NIH 69-97 on February 1, 1971. The surveys of human material for gs interspec reactivity will be intensified.

Significance to Biomedical Research and the Program of the Institute: Reagents and test procedures are being developed for the virion antigens of B- and C-type viruses associated with neoplasia. Detailed structural studies of the purified gs antigens from viruses of several species will provide the information needed to look for similar proteins in man.

Date Contract Initiated:

Current Contract Level: $103,151

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-13)

Title: Studies of Viral-Co-Carcinogenesis

Contractor's Project Director: Dr. Frank Kenney

Project Officer (NCI): Dr. Gio B. Gori

For details of this contract, please refer to NCI-FS-13, Chemical Carcinogenesis Area.

Date Contract Initiated: July 1, 1970

Current Contract Level: $600,000

MICROBIOLOGICAL ASSOCIATES, INC. (NIH-70-2068)

Title: The Roles of Viruses and Chemicals in the Etiology of Cancer

Contractor's Project Director: Dr. Riley Housewright

Project Officer (NCI): Dr. Robert J. Huebner

Objectives:

Project A - Dr. Johng S. Rhim and Project B - Aaron E. Freeman

These projects are concerned with the development, evaluation, standardization and application of in vitro assay systems for studies of carcinogenic agents found in the environment. Test systems include rat, mouse, hamster, and human tissue cultures at advanced levels of subculture, both uninfected and infected with various C-type RNA viruses. Materials under study will include known and suspected environmental carcinogens, and their noncarcinogenic analogs, and DNA tumor viruses.

The focus of Project B is to define the mechanisms of chemical and viral carcinogenesis in vitro, and to correlate these findings with in vivo studies of related programs. The ultimate objective is to develop sensitive in vitro systems for environmental carcinogens.

Project C - Dr. Padman S. Sarma. Development and utilization of sensitive in vitro assay systems for field studies of the prevalence and behavior of the feline leukemia and sarcoma viruses in cat, dog and human cells, including the focus forming, neutralization and complement fixation (COCAL) tests. Emphasis is focused on trans-species rescue of known and postulated defective sarcoma virus genomes from murine, avian, canine and human tumors, using the feline leukemia virus as helper; and on isolation and characterization of endogenous avian, feline and rat C-type RNA viruses/genomes.

Project D - Dr. Carrie E. Whitmire. To a large extent, this project parallels and complements Project A and B. Studies are designed to test RNA virus genome derepression by carcinogens in vivo, leading to gs antigen, infectious virus or tumor induction. The animal systems used include a variety of high and low leukemic incidence strains of mice, hamsters and rats, including those strains from which tissue cultures were derived for Projects A and B.

Project E - Dr. M. Lee Vernon. This project is a continuing one designed to apply electron microscopy of high resolution capability to a search for the presence of C-type virus particles and other viruses and virus-like structures in normal and cancerous tissues and cultured cells. Candidate specimens eminate largely from contract laboratories at Microbiological Associates (NIH-70-2068 and PH-43-67-697), and from the Project Officer.

Project F - Dr. John C. Parker. This laboratory provides the serological support essential to the contract and related (PH-43-670697) programs.

Major Findings:

Project A. Several in vitro systems were developed for sensitive quantitative assay of chemical carcinogens. Findings: Rauscher leukemia virus (RLV)-infected rat embryo cells rapidly transformed by as little as 0.01 ug of DMBA-produced tumors in newborn homologous hosts; control cells failed to show evidence of transformation and produced no tumors.

One of the more promising in vitro methods developed for assaying the relative effects of various environmental carcinogens was the AKR-infected NIH Swiss mouse embryo cell system. This system proved even more sensitive than rat and hamster systems for assaying various experimental carcinogens, and is now being used to test smog and tobacco fractions.

Uninfected and untreated normal rat embryo cell lines exhibited morphological alteration after 65 subcultures and produced tumors in homologous hosts. Concomitantly, a new antigen having the properties of gs antigen, was switched on. Since the antigen, which is one shared with the MSV-induced rat tumor system, was switched on much earlier by cells transformed by polyoma virus, RLV-infected rat cells responded with markedly accelerated transformations when treated with polyoma virus. It therefore appears that cell transformations by polyoma virus, as has been shown with SV40, may be mediated by endogenous or added C-type RNA genomes.

Project B. Dr. Freeman and his group developed several in vitro systems for assay of the transforming effects of benz(a)pyrene, 3-methylcholanthrene, cigarette smoke condensates and smog components. Transformation of a variety of rat and hamster embryo cells with diethylnitrosamine and 3-methylcholanthrene in combination with leukemia viruses, and with city smog by itself and in combination with leukemia viruses, were recently published. Other recent important observations include the following:

When uninfected hamster embryo cell cultures treated with 3-methylcholanthrene or cigarette smoke condensates were transformed and produced tumors in hamsters, 5 of 6 yielded infectious hamster leukemia virus (HaLV), thus indicating that the chemicals activated an endogenous latent HaLV.

Chemical carcinogens on human (WI-38) cells infected with feline leukemia virus (FeLV) have not led to transformations. However, the FeLV-infected, chemically treated WI-38 cells have continued to replicate at normal rates for many subcultures beyond the terminal stages of the untreated control cultures.

Project C. Several new tests for assay of feline tumor viruses were developed by Dr. Sarma and his group, as follows:

(1) A quantitative assay for feline leukemia infectious virus and viral antigen, the COCAL test, modeled after the COFAL test for avian leukosis; and (2) a viral interference test, analogous to the RIF test of Rubin's (avian system), and to a previous test developed by Dr. Sarma for murine leukemia viruses. Preliminary observations have revealed differential interference patterns analogous to those described by Vogt and Ishizaki for the avian leukosis-sarcoma complex. Feline leukemia virus (FeLV) strains and FeLV pseudotypes of MSV derived from these strains can now provisionally be placed in three subgroups: A, B and C. Unlike the COCAL test, the FeLV viral interference test is based on the detection of type-specific rather than group-specific antigens; its usefulness therefore will probably be confined to studies of viral envelope antigens.

Utilizing the new methodology it has been ascertained that the three distinct strains of the feline sarcoma virus fall under subgroup B of the feline leukemia-sarcoma complex.

Dr. Sarma and his group recently isolated a non-cytopathogenic rat-cell-specific C-type virus from stocks of MSV(O). It appears from studies by Dr. Sarma and Dr. Aaronson (VLLB) that this isolate is a rat leukemia virus and that MSV(O), described by Ting in 1968, can be characterized as a rat pseudotype of the Moloney murine sarcoma virus.

A new technique for demonstrating avian leukosis viral genome was also developed during the contract year. A serial tissue culture line of hamster tumor cells derived from a Rous sarcoma virus-induced hamster tumor was found to contain avian C-type virus which proved to be defective. Upon co-cultivation of the hamster tumor cells with cultures of avian embryos positive for avian leukosis gs antigen, the hamster-derived virus acquired the property of oncogenicity for quails. These preliminary studies suggest that the hamster virus is defective requiring a helper virus, supplied in this instance by the vertically transmitted avian leukosis viral genome present in SPAFAS and "clean" University of Connecticut chickens.

Previous studies established and described the prevalence of a vertically transmitted avian C-type viral genome in normal chicken embryos expressed in the form of gs antigen. Follow up studies revealed this antigen to be demonstrable in virtually all embryos at all stages of development, implying not only natural inheritance of the C-type genome, but also that the genome and the gs antigen may play a critical role in avian growth and development.

Project D. Studies initiated in 17 strains of mice during the past year to determine the effects of endogenous C-type RNA viral genome on tumor induction with 3-methylcholanthrene were completed. Histological studies showed that most of the tumors induced subcutaneous fibrosarcomas at the site of infection. Of most interest was the finding that the majority of tumors were positive for gs antigen in the complement fixation test, furnishing evidence that the carcinogenic chemicals "switched on" the RNA tumor virus genome in the induced tumors. In additon to pointing up differences in tumor/virus/antigen expression, determined by the genotypes of the inbred mice studied, it is not surprising to note that variations in response between the inbred strains was greater than in non-inbred mouse lines, thus reflecting the comparatively greater influence of gene segregations in the former which are not observed in the presumably more stable gene pools of continuously outbred strains. (An interesting but not unexpected disparity was noted in the high leukemic strains which are programmed for thymic lymphoma and hence are resistant to induction of fibrosarcomas by hydrocarbons. The mutual exclusiveness between lymphomas and sarcomas induced by chemicals in these studies was similar to that observed in the HRS/J strain studied by Drs. Meier and Myers at the Jackson Laboratory).

The results in these studies have established baselines for developing sensitive in vivo assay systems for measuring the carcinogenic potential of the environmental carcinogens also under test in vitro in Projects A and B.

Project E. More than 1,000 specimens were received for electron microscopic study from the programs on this contract and related collaborating groups (Viral Carcinogenesis Branch, Ph-43-67-697, PH-43-67-744). The most important controlled study done consisted of a systematic survey for C-type and other "viral" particles in embryo tissues from a number of strains of mice at various gestational periods. The results indicated widespread distributions of budding forms of C-type RNA viruses in prenatal tissues, findings which correlate with results in complement fixation. The particles were associated principally with rapidly growing tissues such as the hematopoietic organs. These data confirmed previous evidence that the C-type RNA viral genome is part of natural inheritance in mice and may play a regulatory role during embryogenesis.

Parallel studies of uterine tissue to determine the influence of age, gestational stage, number of pregnancies, coitis and conception on the distribution of C-type particles have shown a widespread distribution, again suggesting a regulatory role in rapidly growing tissues.

Recently initiated studies of mouse ova and the preimplantation stages of mouse embryos by complement fixation and electron microscopy represent an area of increasing effort. In addition, studies patterned after those conducted in the mouse are being extended to selected strains of other species, including the chicken, guinea pig, rabbit and hamster.

Project F. The serodiagnostic laboratory has been in operation for almost a year. This laboratory primarily services Project D of this contract and contract PH-43-67-697 and has a capacity of approximately 2,000 tests per week.

Proposed Course: Projects A and B. Studies will be continued on the mechanism of carcinogenesis *in vitro*, particularly (1) activation of endogenous C-type viral genomes; (2) chemical activation of viral oncogenes; and (3) the effect of a variety of DNA oncogenic viruses in transforming cells uninfected and infected with RNA tumor viruses. The ultimate objective is to develop reliable, sensitive and rapid assay systems for environmental carcinogens.

Project C. The development and refinement of tests for detection of endogenous mammalian oncogenes for eventual application in the human system will be continued.

Project D. With the establishment of baselines of C-type RNA virus and viral genome expression in selected mouse strains, large scale *in vivo* assays of a variety of known and suspected environmental carcinogens will be undertaken; these tests will be run in parallel with materials to be tested and cell lines utilized in Projects A and B.

Project E. Electron microscopy studies of various tissues associated with rapid cell replication (embryos, ova, etc.) will be expanded; preliminary studies of human cancer tissue will be continued at an increased level.

Project F. The serodiagnostic laboratory will be utilized to full capacity, and will likely service additional contract and inhouse operations in the near future.

This contract program is an integral part of the Viral Carcinogenesis Branch research effort.

Date Contract Initiated: February 1, 1970
(Note: On February 1, 1970, the following contracts were combined into the existing contract: PH-43-64-941, Ph-43-68-705 and PH-43-68-706).

Current Contract Level: $2,080,475

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-7)

Title: Molecular Anatomy Program, NCI Segment

Contractor's Project Director: Dr. Norman Anderson

Project Officer (NCI): Dr. Charles W. Boone

Assistant Project Officers (NCI): Dr. Robert A. Manaker
Dr. Timothy O'Connor

Objectives: (1) To study the role of embryonic antigens in tumor immunity, (2) to screen for the presence of embryonic antigens in human tumors, (3) to isolate and characterize tumor-specific transplantation antigens, (4) to apply various in vitro assays for humoral and cellular immunity to the longitudinal study of human cancer patients, (5) to develop new sensitive methods for assaying immune reactions, and (6) to design and manufacture improved apparatus for preparative electrophoresis in chromatography.

Major Findings: In the last year, by far the most outstanding finding was that hamsters can be protected against challenge with syngeneic SV40 transformed tumor cells by prior immunization with fetal tissue which has been x-irradiated to prevent further development after inoculation. Fetal tissue from mice also protected against tumor challenge. The SV40 virus-induced tumor-specific surface antigen of hamster tumor cells was successfully isolated and shown to be active by isograph rejection. A new and highly sensitive method for measuring antibodies in virus particle concentrations was developed, involving the binding of the antibodies or viruses to latex beads which are separated on density gradients.

Significance to Biomedical Research and the Program of the Institute: If the finding is confirmed that immunization of the adult animal with early embryonic tissue protects it against tumor challenge, a whole new era in cancer research will be open: the role of embryonic antigens in tumor immunity. The potential significance of this area is in revealing mechanisms of carcinogenesis and in suggesting new avenues for immunodiagnosis and immunotherapy.

Proposed Course: Efforts will concentrate on confirming the fundamental finding that animals can be immunized with fetal tissues against tumor challenge. Efforts will continue on developing improved in vitro assays for humoral and cellular immunity.

Date Contract Initiated: July 1, 1962

Current Contract Level: $674,000

LIFE SCIENCES, INC. (PH43-68-711)

Title: Production of Germfree and Reagent Grade Specific-Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar
Dr. W. Ray Bryan

Objectives:

(1) To maintain foundation colonies of germfree and SPF animals of species and strains required for program as designated by the Project Officers.

(2) To produce animals for research, both germfree and specific-pathogen-free (SPF) derived therefrom. The latter are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic micro-organisms or infestation by parasites and are referred to as "reagent grade" hosts (RG-SPF).

(3) To transport or ship such animals to SVCP participants in germfree equipment as designated by program management through the Project Officers.

Major Findings:

(1) An inbred germfree colony of BALB/c mice is maintained as a foundation colony to furnish breeders for replacement of retired breeders of the SPF production colonies.

(2) RG-SPF colonies of BALB/c mice, derived from germfree foundation colonies, have been established in separate environmental control cubicles as follows:

a. Inbred RG-SPF colony for producing pedigreed mice for research.

b. Random bred RG-SPF (non-pedigreed but derived from BALB/c foundation stock) for producing mice for general purposes such as assays, virus passages, production of reagent grade virus for research, etc.

Both colonies have been tested and certified free of all common murine viruses tested for, except leukemia virus which is transmitted vertically and is inherent in all known mouse stocks.

(3) A SPF colony of NIH mice (Swiss) is maintained as insurance for the preservation of this important stock. Limited numbers of animals are supplied to intramural scientists of NCI.

(4) An RG-SPF laying flock of Japanese quail (Coturnix coturnix) has been established from initially germfree stock and is now producing

eggs for research and hatching at a rate of about 700 per week.

(5) Germfree quail are being produced in limited numbers, as required for SVCP programs, by surface sterilization of eggs from the RG-SPF laying flock (see (4)) and hatching germfree.

(6) A foundation colony of leukosis-virus free White Leghorn chickens (LSI-C) derived from the University of Connecticut (Luginbuhl) stock is now in the third generation since establishment from germfree chicks. It is maintained by pedigreed (random bred) pairs of male and female birds behind a tertiary barrier for environmental control. All birds in the foundation colony are monitored periodically both for leukosis virus and extraneous viruses, and embryos from this pedigreed source are continuously monitored for freedom from leukosis virus expression. Only fertile eggs from certified virus free pairs are used for maintenance of the expansion and production colonies which supply eggs and chicks for research.

(7) Expansion and production colonies of LSI-C reagent grade chickens are maintained behind secondary-barrier protected facilities. All space that is now available under the current contract is being used for producing fertile eggs and chicks for Marek's disease research under another contract (PH43-69-63) with this contractor. The production rate is at about 350 eggs (or chicks) per week.

(8) A viral testing laboratory is being operated for the monitoring of all avian animals in the foundation and production colonies maintained under this resource contract.

Significance to Biomedical Research and the Program of the Institute: This contract was established to serve the pressing, essential need for developing and maintaining "reagent grade" animals for research on oncogenic viruses. Such viruses may be either interfered with, or enhanced, by other viruses present in simultaneous infections, and reproducible, meaningful, results on oncogenic viruses cannot be obtained in animals harboring varying types or amounts of other adventitious viruses. The foundation stocks developed and maintained under this contract are free of such adventitious viruses and are the prime source of these particular strains and stocks of animals used in various intramural and contractor laboratories participating in the SVCP.

Proposed Course: This service type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of SVCP activities as they occur.

Date Contract Initiated: February 8, 1968

Current Contract Level: $279,500

COLUMBIA UNIVERSITY (NIH-70-2049)

Title: RNA and RNA Replicases in Tumor Cells Associated with RNA Oncogenic Viruses

Contractor's Project Director: Dr. Sol Spiegelman

Project Officer (NCI): Dr. Timothy E. O'Connor

Objectives: To explore the mode of replication of RNA-containing oncogenic viruses and the molecular mechanism(s) underlying viral carcinogenesis. Studies are concerned with the characterization of both viral and host enzymes involved in oncogenesis and, in particular, with the enzymes present in purified virions.

Major Findings: An RNA-dependent DNA polymerase (RIDP) has been demonstrated in a variety of purified Type C (RSV, Twiehaus Agent, AMV, RLV, MSV, FeLLV, FSV) and Type B (Mouse MTV) oncogenic virions, as well as in the Mason-Pfizer Virus, but not in a variety of typical non-oncogenic RNA-containing viruses (NDV, A and W influenza, Reo, VSV and Polio). Polymerase was found in the RNA containing Type C Visna Virus which is the causative agent of a neurological disease in sheep. Visna Virus was found to resemble the Type C oncogenic virions in that synthesis of DNA is mandatory for productive infection of cells.

The presence of RIDP in purified virions was found to be invariably accompanied by a second enzymatic activity--DNA dependent DNA Polymerase (DIDP). DIDP, which has the same substrate requirements as RIDP, was clearly demonstrated by enzymatic destruction of the RNA content of the ruptured virion followed by synthesis of specific DNA in response to addition of exogenous DNA template. DIDP, unlike RIDP, is inhibited by actinomycin D. Unlike the previously known cellular DNA polymerases, DIDP shows a preference for double-stranded rather than single-stranded DNA as template, and it is postulated that the enzyme is probably involved in conversion of the DNA-RNA hybrid product of RIDP to double-stranded DNA product.

Using synthetic oligonucleotides as templates, it was established that oncogenic viruses contain DNA polymerase activities directed by single-stranded RNA, double-stranded RNA, double-stranded DNA, and DNA-RNA hybrids. Certain synthetic templates were found to be superior to natural templates by almost two orders of magnitude in stimulating polymerization. The number of different kinds of protein molecules required to handle this variety of templates is under investigation. Enzyme(s) from AMV and virus-infected avian myeloblasts has been solubilized and purified by several orders of magnitude using a sequence of velocity gradient centrifugation and column chromatography on DEAE-cellulose and CM-Sephadex. The apparently homogenous product which migrates as a single entity on acrylamide gels is dissociated into components on SDS-acrylamide gels. The enzymatic properties of each component is under study.

The buffy coats of 120 leukemic patients (ALL, CLL, AML, CML, monocytic, chronic granulocytic and leukosarcomas) and 70 normal patients as well as white blood cells from patients with leukoproliferative diseases other than leukemia (polycythemia vera, myeloid metaplasia and various leukocytoses) were tested for the presence of a DNA polymerase that responds to the synthetic templates dC:dG and DT;rA. Except for two monocytic leukemias, all enzyme preparations from leukemics were positive, whereas all preparations from control normal or "blood discrasia" patients were negative. When enzyme levels were followed through periods of chemotherapy, dramatic drops in enzyme activity were observed when patients entered remission. The enzyme(s) from human leukemic cells is now being characterized.

DNA polymerase activities that respond to the synthetic templates dC:dG and dT:rA have been found in embryonic tissues of chickens, mice, rats and humans. These activities are highest during early periods of development and fall as embryogenesis progresses. The experience accumulated to date with cell lines suggests that every proliferating cell has an elevated response to dC:dG. Not all, however, show responses to dT:rA and rA:rU. The proteins responsible for enzymatic activity in embryonic, leukemic, and cancer cells are now being purified and compared.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the molecular mechanisms involved in oncogenic viral replication and in cellular transformation may ultimately provide a rationale for effective control of neoplasia. Research by contractor and other investigators on "reverse transcriptase" during the current period has added a new chapter in the knowledge of genetic processes in mammalian cells. The polymerase activity of extracts of human leukemic cells as compared to normal lymphocytes may provide a useful diagnostic tool for human leukemia.

Proposed Course: Studies on the characterization of viral and host enzymes involved in viral replication and oncogenesis will actively continue.

Date Contract Initiated: October 29, 1969

Current Contract Level: $400,000

MELOY LABORATORIES (PH-43-66-458) formerly Mel-Labs, Inc.

Title: Murine Mammary Tumor Virus Studies

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Louis R. Sibal

Objectives: Part A is a continuation of the present contract to propagate, purify and provide murine mammary tumor virus (MTV) suitable for collaborating investigators; to perform immunological and biological assays for the detection and quantitation of MTV; to develop improved methods for the propagation and detection of MTV and MTV antigens; to conduct studies on the control of neoplasia in the susceptible murine host by vaccination with inactivated virus. The ultimate objective of this part is to apply information to the study of the possible viral etiology of breast cancer in man.

Part B. The contractor will increase production of MTV positive-mouse milk to prepare much needed purified virus for inhouse and contract laboratories.

Major Findings: C_3H milk production has been increased significantly. The use of programmed sucrose density gradients in the rate-zonal step of virus purification has resulted in better separation of the virus band from high and low density protein bands. Greater yields of purified virus have resulted from the use of these new gradients.

Hemagglutination, hemagglutination inhibition, immunodiffusion and indirect fluorescent antibody assays have become reliable, reproducible, and sensitive testing procedures for viral antigens and/or antibodies to MTV.

A series of experiments to determine whether vaccination of C_3H with purified, formalin-inactivated MTV has been initiated. Immunized mouse groups showed humoral antibodies to MTV; milks collected from females producing a second litter are now being assayed for viral antigen. Data relating to protection from tumor development will be available for the next report.

Investigations to establish in culture, a cell line which produces significant amounts of MTV *in vitro* and to develop an *in vitro* assay system for MTV have been initiated. To date, 19 tumor cell suspensions have been established in tissue culture. Of these, 10 lines were free of mycoplasma and bacteria and are positive for MTV antigens by immunofluorescence. Cells growing in the absence of hormonal stimulation are almost entirely fibroblastic; cells growing in the presence of insulin hydrocortisone and prolactin at a final concentration of 10 ug/ml are epithelial in nature. On the basis of serological and electron microscopic tests, it is very likely that a considerable amount of MTV or MTV antigens were synthesized *in vitro* by hormone stimulated cells.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. The recent finding of a virus, resembling a Type B RNA oncogenic virus of mice, in the milk of a significant number of women from high-risk breast cancer families strongly suggests a possible viral etiology for this disease. A major effort of the Special Virus Cancer Program will be directed to determine the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in man may be developed.

Proposed Course: The biological and immunological methods developed in this laboratory will be used in systematic studies to develop further the mouse MTV system as a laboratory model for breast cancer virus studies in man. Greater emphasis will be placed on propagating this virus in tissue culture and on *in vivo* infection of cells in cultures. It is anticipated that the information gained from these studies will be applied to human breast cancer studies within the newly-created Breast Cancer Virus Segment.

Date Contract Initiated: December 30, 1965

Current Contract Level: $175,356

RUTGERS, THE STATE UNIVERSITY (PH-43-68-1025)

Title: Cross Protection Studies Among Murine Leukemia Viruses

Contract's Project Director: Dr. Nicholas C. Palczuk

Project Officer (NCI): Dr. Louis R. Sibal

Objectives: 1. To immunize Balb/c mice with killed murine leukemia virus vaccine prepared from available representative strains and to determine whether mice are protected from leukemia following challenge by virus from the strain used for immunization as well as each of the other strains.

2. To determine whether a susceptible strain of mice (C57BL/K) can be protected from radiation induced leukemia by the administration of a killed virus vaccine before x-irradiation.

3. To determine whether a strain of high leukemic mice (AKR) can be protected from spontaneous leukemia by the administration of a killed vaccine prepared from virus isolated from this or other strains.

Major Findings: During the course of this study the data have indicated that: (a) mice immunized with Rauscher leukemia virus are protected only against Rauscher and Friend leukemia virus challenge; (b) mice immunized with Friend virus are protected only against Friend and Rauscher virus challenge; (c) mice immunized with Moloney virus are partially protected against Friend and Rauscher virus challenge; (d) mice immunized with Gross leukemia virus were not protected against Rauscher, Friend, Moloney, Buffet, Breyere-Moloney, or Gross virus challenge; (e) mice immunized with Breyere-Moloney virus are currently partially protected against Breyere-Moloney virus challenge; (f) mice immunized with Buffet leukemia virus currently are partially protected against Friend and Buffet virus challenge.

Immunization of female AKR mice with a killed Gross virus vaccine did not completely protect against spontaneous leukemia, but the immunization procedure did succeed in delaying the leukemic time-to-death.

Significance to Biomedical Research and the Program of the Institute: The major goal of the SVCP is to control leukemia in man either by the use of vaccines or other methods. Several techniques used to approach this goal have resulted from laboratory experimentation with murine leukemia viruses. The development of an effective vaccine against murine leukemia would thus serve as a model for a human vaccine at a time when this becomes feasible.

Proposed Course: The work on this contract is nearing completion. The data from these experiments will be analysed when the final report is submitted.

Date Contract Initiated: June 24, 1968

Current Contract Level: $22,676

BIONETICS RESEARCH LABORATORIES (NIH-69-2160)

Title: Support Services for the Special Virus Cancer Program

Contractor's Project Director: Dr. Robert C. Y. Ting

Project Officer (NCI): Dr. George Todaro

Objectives: To provide a laboratory that will collect, process and test cancer specimens from human and animal sources suspected of containing a virus associated antigen.

Major Findings: Support services to the Special Virus Cancer Program included a variety of efforts. An additional 1,250 aliquots from 177 specimens were stored in the serum bank, making the current inventory of serum and plasma about 1,500 specimens divided into more than 5,000 aliquots. Upon request from SVCP investigators, the laboratory was involved in the collection and distribution of 50 tissues and 575 sera to other laboratories for biochemical, immunological and virological studies. Immunological services were also performed for several NCI investigators.

Studies in identical twins were pursued to detect an antigen in the leukemic twin using his normal twin as control. An antigen which appeared to be a leukemia associated antigen was detected in five of the seven sets of identical twins.

The role of EBV in human lymphoma was evaluated by immunological techniques detecting humoral and cellular immunity to the virus. The importance of careful clinical evaluation was emphasized by a study of twenty American patients with Burkitt's lymohoma and age and sex matched controls. Treatment and prognosis correlated with EBV titers in both lymphoma and leukemia patients.

Serological studies with the BeLev cell line demonstrated complement fixation antibodies in 43 (42%) of 102 unselected patients wit breast cancer, most of whom were studied at the time of diagnosis. Only 7 (13%) of 54 patients with benign breast disease and 15 (13%) of 112 patients with other forms of cancer demonstrated CF antibodies to the same cell line. Normal blood bank donors (2 out of 55) had an even lower incidence of antibody.

Dr. Helene Smith (Bionetics) and Dr. Charles Scher (NCI) have developed a very sensitive assay for a serum factor which induces cellular DNA synthesis in much the sam way the tumor viruses induce cellular DNA synthesis.

Significance to Biomedical Research and the Program of the Institute: This contract laboratory provides an opportunity for a systematic, large-scale effort to detect viruses and/or viral antigens in human tumor materials (particularly leukemias and sarcomas), using tissue culture, immunological, biochemical and EM techniques. This is a major objective of the SVCP.

Proposed Course: It is proposed that this contract will continue to supply the necessary supportive services required to meet the needs of the SVCP.

Date Contract Initiated: June 27, 1969

Current Contract Level: $942,000

MELOY LABORATORIES (NIH-70-2047)

Title: Cell Biology Facility

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. George J. Todaro
Dr. Charles W. Boone

Objectives: Part A: To study spontaneous and virus-induced neoplastic transformation by the following methods or projects: (1) development and characterization of 3T3 and 3T12 lines from inbred mouse strains; (2) radiation-virus co-carcinogenesis; (3) elucidation of the differences between the normal and transformation prone human cells; (4) characterization of MSV transformed Balb/c3T3 cells and nonproducer cells; (5) development of assay systems for use in tumor virology; (6) biochemistry of enzymes in tumor viruses; and tumor cells to search for C-type and B-type RNA viruses in human cells.

Part B: Influenza virus-mediated tumor immunity; (2) screening of human cancer specimens for tumor-specific antigens.

Major Findings: Part A: 1. The development of continuous, contact-inhibited mouse cell lines from Balb/c and NIH/Swiss embryo cells has provided excellent model systems for study of the effect of tumorigenic viruses both *in vitro* and *in vivo*. These cell lines are supplied to numerous investigators throughout the world, and are becoming the standard cell lines for biochemical and biological investigations of cellular growth control mechanisms.

2. A study of the effect of UV irradiation of the DNA tumor virus, SV40, on the survival of its viral functions in mouse and human cells has revealed that the viral functions for T antigen and transformation show comparable UV sensitivities but that the virion or V antigen is much more UV sensitive. The UV survival of SV40 functions in normal cells and cells from patients with xeroderma pigmentosum (XP) have been compared. In this disease, cells lack the ability to repair UV damage to cellular DNA. The results show that normal cells have the ability to repair UV damaged virus.

3. A large scale screening program is underway to test human fibroblast cells from patients with a variety of diseases associated with a high incidence of tumors for their susceptibility to transformation by SV40. More than 900 individual specimens have been explanted into tissue culture for testing.

4. A new type of MSV-transformed cell, the nonproducer cell, has been discovered. Such cells are morphologically transformed and are tumorigenic yet lack cell known antigens of the murine sarcoma-leukemia complex. The sarcoma genome can be rescued by the additon of "helper" leukemia virus.

5. A quick screening method has been developed utilizing sucrose gradient analysis of culture fluids from cell cultures to detect mycoplasm contamination. This assay is simple and at least as sensitive as the more time consuming culture methods.

6. Methods have been developed which greatly increase the sensitivity of detection of the viral RNA dependent DNA polymerase using synthetic templates with manganese as the divalent cation. It has been possible to disrupt the tumor virus and to partially solubilize it. Polymerase activity is associated with the internal core of the virus. Certain anti-sera to RNA tumor viruses inhibit the polymerase and studies to date indicate that these sera contain an antibody which inhibits the viral polymerase of several mammalian C-type RNA viruses.

7. Syncytium-forming ("foamy") viruses of several species including the primate, bovine, hamster and feline have been found to contain an RNA dependent DNA polymerase. A thorough understanding of this previously neglected group of viruses is now clearly warranted and studies to define their pathologic, antigenic and biochemical characteristics are in progress. Attempts to find isolates of such viruses from human tissues are also underway.

8. Recently developed biochemical methods to study the viral RNA dependent DNA polymerase in virions have been extended to search for evidence of such enzymatic activity on cells using the model system of MSV transformed and normal Balb/c 3T3 cells. It has been possible to detect

enzyme activity in normal as well as transformed Balb/c 3T3. In addition, normal human fibroblasts contain polymerase activity. The relationship between the enzymes in normal cells, tumor cells and tumor viruses provide the major focus for present studies.

9. Inhibitors of the tumor virus polymerase are being tested for their ability to inhibit virus replication and transformation. Certain rifampicin derivatives have been found to be 50-100 times more active than N-demethyl rifampicin in vitro assays.

Part B: 1. A fundamental experimental finding was that mice can be made immune to tumor cell challenge by vaccination with diluted frozen and thawed homogenates of syngeneic tumor cells provided they have first been infected with influenza virus. Homogenates of uninfected cells were without effect. Homogenates treated with formaldehyde to inactivate the pathogenicity of the virus were shown to be still effective in inducing tumor immunity; (2) The paired label radioantibody adsorption assay, which accurately measures antibody binding to the surface of viable cells, was used to measure AKR virus-induced surface tumor antigens. Techniques were developed so that human leukemia cell surface antigens cross-reacting with AKR virus-induced cell surface antigens can be detected; (3) Solid human tumors are being disaggregated and used in an in vitro assay for autologous antibodies to tumor cells.

Significance to Biomedical Research and the Program of the Institute:
Part A: These studies offer the possibility of determining the sequence of events that occur when a normal cell is converted into a neoplastic cell, suggest a possible simple test for "cancer proneness" in man which would permit recognition of a "high risk" group for cancer in the human population and provide further information at the cellular and molecular level on the "oncogene theory" of cancer.

Part B: The outstanding implication of the finding that influenza virus-infected tumor cell homogenates can protect against tumor challenge is that it raises the possibility of being able to reduce the metastatic spread of cancer in human patients by vaccinating them with influenza virus-infected homogenates of their primary tumor.

Proposed Course: Part A: Effort on this contract will remain essentially the same.

Part B: Work will continue to concentrate on exploiting the phenomenon of influenza virus-mediated enhancement of tumor immunogenicity as it can be applied to animal models for immunotherapy. The mechanism by which this phenomenon occurs will continue to be intensively studied. The contract will continue to provide back-up facilities for basic investigations in the immunology of viral carcinogenesis.

Date Contract Initiated: May 25, 1965

Current Contract Level: Part A - $942,000
Part B - $150,000

CALIFORNIA, UNIVERSITY OF (PH-43-63-13) and NCI-FS-8

Title: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses

Contractor's Project Directors: Dr. Stewart Madin
Dr. Neylan Vedros
Dr. Adeline Hackett
Dr. Walter Nelson-Rees

Project Officer (NCI): Dr. James T. Duff

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biomedical Research Laboratory (NBRL), in Oakland. The program of the CCL is funded by a contract (43-63-13) between the University of California and the NCI. In addition, maintenance and operating expenses generated by the CCL are repaid to NBRL by an interagency transfer of funds (FS-8) between NCI and NBRL. The research studies include the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karotyping of cell cultures, and performing biophysical, virological, and cytogenetic research.

Major Findings: Approximately 40 human breast tumors were cultured through a minimum of 5 passages. These were surveyed for virus particles by electron microscopy and by isopycnic centrifugation for isotope-labelled particles harvested from the culture fluids. No viruses of the C- or B-type were found by either method. Various other human tumors were similarily surveyed. The incidence of herpes-like viruses increased with passage, but no C-type viruses were detected. Long-term cultivation of selected cultures with and without hormones continues in efforts to reveal C-type RNA virus expression.

The Mason-Pfizer Monkey (M-PM) virus-shedding culture is being studied extensively; the virus produced has been biophysically characterized, and comparative studies with 2 serotypes of monkey foamy virus are in progress. The M-PM virus has been successfully transmitted to a cell line developed from a chimpanzee lung.

Cultures derived from adult and embryonic tissues of the HRS/J hairless hr/hr mutant mouse and the haired +/+ mouse have been established. Characterization of the cell lines and the virus being produced by them is in progress.

Studies on the biological properties of the MSV-MuLV complex have shown that high yields of infectious virus can be produced in a high passage mouse embryo cell line. The focus forming assay of MSV-MuLV has been compared with the XC assay in 3T3 Balb/c cells. The addition of 40/ugms/ml of polybrene was found to increase the titer by approximately 30 fold. Ten clones with chromosome markers selected from the XC cell line have been tested for their sensitivity as indicators for MuLV infected cells. The clones have similar levels of sensitivity.

Two cell lines derived from mammary tumors of C3H and Balb/c Balb/cfC3H mice have been in continuous cultures for 20 and 25 months respectively. Both lines are tumorigenic in isologous mice, producing tumors similar to MSV-induced rhabdomyosarcomas. Both cell lines have continuously produced particles typical of the type C oncornaviruses; in addition, the C3H line also produces intracytoplasmic type A and mature type B particles characteristic of the mouse mammary tumor virus. Fluorescent antibody straining has revealed MTV-specific antigens in 1% to 5% of cells in both lines. Complement-fixation and immunopreciptin tests have detected antigens of the murine leukemia sarcoma complex in both lines.

During the period of October-January, a total of 227 cell cultures were distributed to 26 different investigators collaborating with the SVCP. Various cell lines submitted by SVCP laboratories continue to be monitored for chromosome alterations.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; attempt to isolate and characterize viral agents from human tumor cells; continue a reference laboratory karology of cells in culture; study oncogenic viral antigens during embryogenesis and continue basic research in the biology of tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The contractor has developed an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators and SVCP contract laboratories. These studies are oriented toward a study of the fundamental biology of tumor cells, and the interaction between tumor cells and viruses of oncogenic importance.

Date Contract Initiated: October 1, 1962

Current Annual Level: $336,307 (PH-43-63-13)
37,685 (FS-8)

THE JACKSON LABORATORY (PH-43-67-744)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host-Gene Control of Their Expressions

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To determine the natural occurrence of leukemia and sarcoma viruses and viral genomes utilizing inbred, hybrid and recombinant mouse strains to more precisely define the genetic mechanisms underlying mutations, differential leukemogenic responses, and regulation of cell turnover in vivo. Studies are designed to determine the relationship between the C-type RNA virus and host genes associated with spontaneous and carcinogen-induced tumors, and the role of the C-type genome in embryo development and definition of the kinetics of growth of embryonic cultures.

Major Findings: The program to date has played a fundamental role in defining the natural history of the C-type RNA virus and viral genome, contributing significantly to the development of new concepts and approaches in the Viral Carcinogenesis Branch program effort. Specifically, in large measure as a result of this contract effort, it has been established that (1) the C-type virus genome is universal in all mice and very likely in rabbits as well; (2) the presence of infectious leukemogenic or sarcomagenic viruses is a rare event in most mouse strains, and probably appears only as the result of intense inbreeding reflecting a "throwback" to its early genesis; (3) that when it is present, infectious virus is most frequently symbiotic and does not threaten species survival. In addition, there is no apparent horizontal spread of virus.

Dr. Meier and his group have defined a number of genes associated with enhance oncogenicity and have evidence that host susceptibility to neoplasia is very likely attributable to a combination of particular alleles at several loci.

Proposed Course: In addition to ongoing studies, efforts are now being focused on converting high cancer susceptible mouse strains to resistant strains, as well as the reverse, through genetic manipulation. Experiments are being designed to determine the number, mode of inheritance (dominant or recessive) linkage relationships and location of host genes which serve as oncogenic determinants.

Significance to Biomedical Research and the Program of the Institute:

This program has contributed many of the important keys and much of the basic data on the genetic determinants of oncogenesis and the natural expressions of the endogenous C-type RNA viruses and viral genomes. It has pointed up the overwhelming influence of genetic predisposition in the development of natural cancer and in susceptibility to environmental carcinogens. Contractor is well on the way to defining and locating some of the genes and loci involved in oncogenesis and viral replication. Information derived from this research bears direct relevance to the human cancer problem and, in addition, may well provide important leads to the autoimmune phenomenon and the role of the C-type genome in normal mammalian growth and development.

Date Contract Initiated: May 2, 1967

Current Contract Level: $299,010

MICROBIOLOGICAL ASSOCIATES, INC. (PH-43-67-697)

Title: Detection, Characterization and Assay of Animal Tumor Viruses

Contractor's Project Director: Dr. Robert M. Nims

Project Officers (NCI): Part I - Dr. W. Ray Bryan
Part II - Dr. Robert J. Huebner

Objectives: Parts I and II

Part I - This project provides essential service type activities, including in vivo bioassays of leukemogenic and other RNA tumor viruses, histopathology, inventory and storage of human and animal specimen materials, and other services involved in various intramural and extramural collaborative SVCP activities.

Part II - This project utilizes in vitro methods for detection and assay of the murine C-type RNA virus and viral genomes in spontaneously occurring tumors and leukemias as well as in normal and embryonic tissues of mice, to determine the possible role of the C-type genome in normal growth and development as well as its association with the neoplastic process.

Major Findings:

Part I - The routine assay of RNA tumor viruses in animals (a cumbersome and costly procedure) has been reduced to about half that of the previous year because of the development under Project II of the XC test, a sensitive and efficient tissue culture method of assay. This advance is reflected in a marked decrease in the number of mice used, making available animal holding space for introducing new strains of mice and hamsters for an expansion of research studies under Part II.

More than 15,000 pregnant SPF mice were produced for NCI program use; 120 pregnant mice per week are being furnished to intramural laboratories. Extensive histopathology services were provided to 14 different investigators or laboratories as needed.

Part II - As noted above, development of a highly efficient test for viral bioassay (the XC test). Since its inception, well over 7,000 spontaneous mouse tumors and 255 rat tumors have been studied in a variety of tests and experimental circumstances under this project.

Cell-free extracts from 47 of 150 spleen and tumor specimens from mice with spontaneous neoplasms yielded 10+ times the expected rate of spontaneous tumors in mice; the most common neoplasms induced were those which occur at the highest rates spontaneously, including lymphocytic leukemias, reticulum cell sarcomas and mammary tumors.

Preliminary experiments in an inbred Balb/c Cr colony on the effects of gene segregation on C-type RNA tumor virus gs antigen and tumor expressions revealed marked differences within several generations. Correlations between antigen expression and tumor development will be undertaken as tumors develop.

Concerted efforts to rescue defective sarcoma virus from spontaneous mouse and rat tumor cell lines proved unsuccessful. It appears from these and related studies that the presence of infectious virus is unnecessary and a rare event in naturally occurring cancer.

Embryonic specimens were found to be strongly positive for gs antigen at various stages of development; as expected, the largest number of positives and highest titers were found in hematopoietic and other rapidly replicating tissues. Parallel studies of maternal tissues revealed high levels of antigen expression in the ovary, uterus and mammary gland. These results are being extended and confirmed in ovariectomized mice treated with estradoil. Results to date add confirmatory evidence to the hypothesis that the C-type viral genome plays a critical role in normal growth and development as well as in the neoplastic process.

Significance to Biomedical Research and the Program of the Institute:

Part I - This contract represents an extension of the laboratory facilities of NIH scientists in the Special Virus Cancer Program who need the services of bioassay systems for oncogenic RNA viruses and histopathology, thus saving an enormous amount of time, space and job positions. It is also used by contractors within the Special Virus Cancer Program.

Part II - This is a direct extension of the Viral Carcinogenesis Branch program to develop *in vitro* detection and assay systems applicable to the human cancer problem. The demonstration of a C-type virus genome present in all cells with an apparent role in the developing embryo reveals the cancer process to be a natural biological event. The ability of the host to repress expression of the genome during most of its lifetime and the factors influencing expression and repression represents a promising approach for the control of cancer, and the Balb/c strain would appear to be a particularly appropriate model for such studies.

Proposed Course: It is anticipated that both parts of this contract will continue indefinitely to back up the activities of the SVCP and to supplement the Viral Carc-nogenesis Branch program. Long-term longitudinal studies will continue wit- stress on the role of the C-type RNA genome in normal growth and in naturally occurring cancer. In addition, more definitive studies on the influence of gonadotropic hormones on genome expression will be undertaken.

This facility will also provide the animals and experienced personnel to help isolate and test postulated natural inhibitors and/or repressors of the RNA virus oncogene, and also of such synthetic or exogenous anti-neoplastic agents that may become available.

Date Contract Initiated: November 15, 1961

Current Annual Level: $731,200

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-66-914)

Title: Establish and Operate a BALB/c Mouse Colony

Contractor's Project Director: Mr. Wilbur Athey

Project Officer: Mr. Samuel M. Poiley

Objectives: To provide BALB/cAn mice for laboratory investigations supported by the SVCP, primarily for virus bioassays on Contract 43-67-697.

Major Findings: The contractor has provided the maximum numbers of mice that can be produced in 2,500 cages. All regulations of The Institute for Laboratory Animal Resources are followed carefully. Requests for animals based on age, sex, weight, suckling litters, or breeders, etc. have been consistently met.

Significance to Biomedical Research and the Program of the Institute: The availability of high quality BALB/cAn mice for biological assays of murine tumor viruses is important to the goal of understanding the role of viruses in oncogenesis.

Proposed Course: Mouse production will be continued at the current level.

Date Contract Initiated: June 16, 1966

Current Contract Level: $65,000

RESOURCES AND LOGISTICS SEGMENT

Dr. Robert Holdenried, VLLB, VO, ET, Chairman
Dr. George J. Todaro, VLLB, VO, ET, Vice Chairman[1]

UNIVERSITY OF CALIFORNIA AT SAN DIEGO (NIH 70-2202)

Title: Development and Operation of a Breeding Colony of Domestic Cats

Contractor's Project Director: Dr. Alexis J. Kniazeff

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To develop and operate a permanent breeding colony of cats which will supply bred offspring for cancer research.

Major Findings: An outdoor type of colony has been developed with only enough solid walls and roofs to provide shelter from sun, rain and wind. The population consists of 200 adult females, 21 breeding males, and approximately 35 immature kittens. Chorionic gonadotropin has successfully stimulated estrus in about 50 cats.

Significance to Biomedical Research and the Program of the Institute: The colony will supply bred cats to Program for use in the cat leukemia-sarcoma studies.

Proposed Course: Continue operation to provide pregnant cats to cancer research laboratories from an open colony of pound-derived cats, and to build a "closed colony."

Date Contract Initiated: June 25, 1969

Current Annual Level: $110,000

UNIVERSITY OF COLORADO MEDICAL CENTER (NIH 69-2080)

Title: Collection of Pediatric Tumor Specimens

Contractor's Project Director: Dr. William E. Hathaway

Project Officer (NCI): Dr. Paul H. Levine

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[1] Replaced Dr. Robert H. Depue, Jr. in February 1971.

Objectives: To obtain tissues and serum specimens from pediatric oncology patients and suitable controls for collaborative studies with the SVCP.

Major Findings: Tissue, sera, and appropriate clinical information were provided to investigators at NCI; Pfizer & Company, Inc.; Bionetics Research Laboratories, Maryland; and Meloy Laboratories, Virginia. Leukemic cells and pediatric solid tumors were useful for tissue culture, immunological work, and polymerase studies. Follow-up samples from patients and family members provided suitable material for a variety of investigators. Since this contract has been operating primarily as a resource, the major findings are being reported under other annual reports.

Significance to Biomedical Research and the Program of the Institute: This is a major pediatric research contract in the United States, which provides specimens to NCI and East Coast laboratories. Specimens obtained from this contract are being investigated by a number of NCI and SVCP laboratory workers.

Proposed Course: Continue to collect serum and tumor specimens as in the past. Immunological investigations, using fresh lymphocytes and tissues, will be initiated by the contractor. Additional personnel are being made available to collect specimens from adults with cancer, as well as children. In addition, individuals with high risk to neoplasms will be investigated for host factors which increase the possibility of developing cancer. Materials will be sent to NIH for virological and immunological studies.

Date Contract Initiated: June 18, 1969

Current Annual Level: $80,000

UNIVERSITY OF CONNECTICUT (NIH 69-52)

Title: Development and Maintenance of a Specific Pathogen Free Flock of White Leghorn Chickens

Contractor's Project Director: Dr. Roy E. Luginbuhl

Project Officers (NCI): Drs. Robert Holdenried and Roy Kinard

Objectives: Establish and maintain a flock of chickens free of specified pathogens, including avian leukosis viruses, and to provide eggs for research use.

Major Findings: Approximately 14,000 eggs or cell cultures were provided to cancer research. A few of the recipients were Drs. J. Beard, M. Green, H. Morgan, F. Deinhardt, P. Sarma, and G. S. Beaudreau.

There is no serologic evidence of the following organisms or diseases in the SPF flocks: *Mycoplasma gallisepticum* and *synoviae*, *Salmonella pullorum*, Newcastle disease, avian infectious bronchitis (Connecticut and Massachusetts strains), infectious laryngotracheitis, avian encephalomyelitis, CELO virus, and the three serotypes of RSV. There have been no clinical cases of Marek's disease (MD); however, MD-associated herpesvirus (MDHV) antibodies have been detected in some flocks using the agar gel precipitin test. Nevertheless, the prime flock and two of four pens of their progeny remain clinically and serologically free of MD. Thirty-six birds of one flock have been identified as gs antigen negative and nine as gs antigen positive, and separate flocks have been reproduced from these identified birds.

Significance to Biomedical Research and the Program of the Institute: The methods being developed indicate that eggs free of specified infectious organisms can be produced. A significant portion of the avian leukosis research in the United States is dependent on the continued availability of this highly controlled and monitored flock.

Proposed Course: Continued maintenance of the flock, with development of genetic lines of chickens characterized for the susceptibility of their embryos to leukosis virus. Flocks free of MD virus and gs liver antigen are being established.

Date Contract Initiated: June 18, 1962

Current Annual Level: $89,700

FLOW LABORATORIES, INC. (PH43-65-1012)

Title: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens

Contractor's Project Director: Mr. Jack W. Walker

Project Officer (NCI): Miss Marie E. Purdy

Objectives: To provide for the SVCP a centrally located low temperature storage and distribution center for viral reagents and tissues.

Major Findings: In 1970 there was a decrease from 1969 in outgoing shipments from 349 to 312; receipts dropped from 49 to 31. The number of items dropped from 16,458 to 10,220 outgoing and from 38,370 to 26,200 incoming. The heat problem generated by the large number of mechanical freezers has been corrected by installation of air conditioning.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible adequately characterized resource materials. The storage and shipping facilities operated under this contract enable the scientist to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: We anticipate that the repository activities will remain at about this year's level.

Date Contract Initiated: June 22, 1965

Current Annual Level: $61,000

HOSPITAL FOR SICK CHILDREN (PH43-65-97)

Title: Human Leukemic and Normal Tissue Collection and Preservation

Contractor's Project Director: Dr. Peter McClure

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To obtain serum and plasma specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls.

Major Findings: In the past contract year, serum collection was broadened to pediatric patients with other solid tumors, in response to demands from SVCP investigators. The major contribution of the contract, however, was the identification of three leukemia patients who had clinically normal identical twins. The families were investigated at the Hospital for Sick Children, where establishment of identity was confirmed, using fingerprinting, teeth moldings, and mixed leukocyte cultures. The families were also admitted to NCI for thorough virological and immunological studies. Leukemia-associated antigens, possibly virus-related, were identified in two of the three leukemic twins. The one set of twins studied during a two-year remission showed no evidence of leukemia-associated antigens. The contractor has also identified three patients with concurrent infectious mononucleosis and acute leukemia, which has provided additional evidence immunologically for a distinct etiology for the two diseases.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor can respond rapidly to a variety of SVCP needs. Although the shipment of certain specimens is made difficult by problems with customs, by referring patients to NCI for study and by collecting serum samples in which 24 to 48 hour delays are not critical, the contract provides a valuable service to SVCP.

Proposed Course: To continue the virological and immunological studies on the twin families already identified. The contractor will continue to look for other patients of interest, and will continue to collect serial specimens from pediatric cancer patients and family members.

Date Contract Initiated: February 3, 1965

Current Annual Level: $13,500

HUNTINGDON RESEARCH CENTER (NIH 69-54)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officers: Drs. Robert Holdenried and Daniel J. Rubin (NCI), and Wallace P. Rowe (NIAID)

Objectives: To develop, produce, and characterize special diagnostic reagents for use in the SVCP, primarily antisera and antisera conjugates to viruses, GS antigens, globulins of various animal species, and to T-antigens of polyoma and SV40 in tumored hamsters.

Major Findings: The anti-MSV(M) sera from tumored rats produced by this contractor remains, as in the former year, the most widely issued reagent. The anti-AKRV sera from tumored rats has shown inhibitory activity against polymerase. The anti-MSV(M) sera are reactive against interspec antigen and murine leukemias. These reagents are experiencing expanded application in cancer research. Anti-human cell propagated Rauscher leukemia virus (intact and disrupted fractions) sera has been produced and is being characterized. Anti-cat leukemia sera (against intact and disrupted fractions) is also being characterized.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed provide tools used in cancer research. The project functions in close collaboration with SVCP research projects and the Laboratory of Viral Biology, NIAID.

Proposed Course: Continue development, production, and characterization of serological test systems.

Date Contract Initiated: June 2, 1963

Current Annual Level: $140,028

JOHNS HOPKINS UNIVERSITY (NIH 69-2008)

Title: Maintenance of a Flock of RIF-Free Chickens

Contractor's Project Director: Dr. Frederik B. Bang

Project Officer (NCI): Dr. W. Ray Bryan

Objectives: To maintain the small "closed" flock of leukosis-free White Leghorn chickens to supply fertile eggs for use in avian tumor virus studies.

Major Findings: The eleventh generation of the original flock is now in egg production. About five dozen eggs per week are shipped to Life Sciences, Inc., St. Petersburg, Florida, for use in Marek's Disease studies on Contract PH43-69-63. Additional eggs are used in cell culture and avian tumor virus studies at Johns Hopkins University.

Significance to Biomedical Research and the Program of the Institute: This flock supplied the birds used by Dr. Bang in his important studies on avian tumor viruses. The genetic heritage must be maintained to permit the continued definitive research with this highly characterized flock.

Proposed Course: Indefinite

Date Contract Initiated: March 24, 1969

Current Annual Level: $8,600

UNIVERSITY OF LOUISVILLE (PH43-66-902)

Title: Preparation of Simian Foamy Virus Reagents and Antisera

Contractor's Project Director: Dr. Paul B. Johnston

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To prepare and test reference reagents (virus and corresponding antisera) for the simian foamy viruses, types 1-7, and foamy virus from other laboratory species.

Major Findings: The seven types of simian foamy viruses have been prepared and packaged. These are being tested for homogeneity, potency, and purity by Dr. Johnston. He also identifies, and if needed will prepare, reagents for foamy virus from species of cancer research animals other than primates.

Significance to Biomedical Research and the Program of the Institute: The simian foamy virus reagents will be used in the identification of viruses and viral antibodies in primates used for cancer research. The indigenous viruses of laboratory primates pose husbandry problems, in addition to contaminating test systems and complicating the attempts to recover oncogenic virus from tissues and tissue extracts. The specific antisera may also be useful in suppressing the growth of these adventitious viruses in primate tissue cultures. The identity of cat syncytial virus isolated is being determined.

Proposed Course: The packaged foamy virus reagents now in low temperature storage will be checked for titer stability at selected time intervals. This laboratory will also assist in the detection of foamy virus contaminates on a referral basis.

Date Contract Initiated: June 13, 1966

Current Annual Level: $9,952

MAKERERE UNIVERSITY (PH43-67-47)

Title: Epidemiologic Study of Burkitt's Lymphoma

Contractor's Project Director: Dr. George Kafuko

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To conduct studies on the natural history, occurrence, and transmission of Burkitt's lymphoma (BL), with special reference to the etiologic role of Epstein-Barr virus (EBV).

Major Findings: A pilot study for a large scale prospective sero-epidemiologic study of EBV and BL in the West Nile region of Uganda has been completed. This pilot study demonstrated that the immunofluorescent titers to EBV are stable in children, and the cooperation of the population is sufficient to embark on the large scale studies proposed by the International Agency for Research on Cancer.

Significance to Biomedical Research and the Program of the Institute: The SVCP conducts many laboratory studies of EBV. The large scale sero-epidemiologic project is designed to help determine whether this virus is etiologic for BL and whether continued laboratory studies are indicated.

Proposed Course: This contract will cooperate in the proposed large study to determine the possible effect of co-factors with EBV necessary to development of BL.

Date Contract Initiated: September 26, 1966

Current Annual Level: $12,000

MARQUETTE UNIVERSITY (PH43-68-1010)

Title: Effect of Human Pregnancy Hormones on Breast Cancer Cells *In Vitro*

Contractor's Project Director: Dr. Ronald Pattillo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To test the effect of co-cultivation of hormone-secreting human choriocarcinoma cell lines with breast cancer lines in an attempt to increase the yield of a virus detected in human breast cancer.

Major Findings: The contractor has established *in vitro* cell lines from human choriocarcinoma cases. These lines secrete *in vitro* human chorionic gonadotropin, lactogenic hormone, estrogen, and progesterone. One of these lines (BeWo) is being co-cultivated with the Levine 3 human breast cancer line (isolated by another contractor). No increase in the virus yield of Levine 3 has been detected by electron microscopy as yet; however, the morphology and culture characteristics of the line do change. Not all combinations of hormone yield have been tested yet. A test more sensitive to virus production than electron microscopy will be instituted as soon as feasible since EM is a slow and inefficient way to monitor these experiments. BeWo will be used to help isolate new breast cancer cell lines in an attempt to find one that yields more virus.

Significance to Biomedical Research and the Program of the Institute: The virus present in Levine 3 is the prime candidate for a human breast cancer virus, but it has not been isolated in sufficient quantity to use as an antigen to determine its significance and distribution. Therefore, methods to increase the yields are vital to further progress in this area.

Proposed Course: To continue co-cultivation studies, and to attempt establishment of new breast cancer lines.

Date Contract Initiated: September 19, 1963

Current Annual Level: $61,500

UNIVERSITY OF MICHIGAN (PH43-65-639)

Title: Collection of Leukemia-Lymphoma Specimens

Contractor's Project Director: Dr. Chris J. D. Zarafonetis

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To collect and distribute specimens and information from patients with leukemia or lymphoma.

Major Findings: Collection of specimens from this contractor has provided valuable support for SVCP virology studies. Six units of leukocytes from two patients with acute leukemia provided valuable information and source material for polymerase studies carried out by Dr. Stuart Aaronson and Dr. Robert Gallo (NCI), and Dr. S. Spiegelman (Columbia). Buffy coat and white cell rich plasma from six other patients with high white counts have also been provided for polymerase studies. A total of 3,704 serum samples have been collected. Sera, skin biopsies, lymph nodes, and other tissues were distributed to a number of SVCP investigators.

The computerization of the serum bank inventory records is being developed with the consultation of Dr. Deward Waggoner.

Significance to Biomedical Research and the Program of the Institute: Availability of clinical specimens and pertinent information on the cases is paramount in the achievement of a major goal of the SVCP, i.e., to identify, rescue, characterize, and propagate a candidate human cancer virus. Large volumes of leukemic cells are necessary for the biochemical characterization of the polymerases present in these cells, and a number of clinical contracts will be necessary to keep up with the biochemical demand. In addition to helping meet this need, the contractor is also collecting a large number of sera for the

SVCP serum bank and has been able to meet new requests for a variety of specimens for SVCP investigators.

Proposed Course: Continuation as described.

Date Contract Initiated: June 21, 1965

Current Annual Level: $58,720

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-700)

Title: Development of Murine Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. John C. Parker

Project Officers: Drs. Robert Holdenried (NCI) and Wallace P. Rowe (NIAID)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent and cat viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations.

Major Findings: This contract project provides to the NCI a heavily used, highly skilled murine virus diagnostic laboratory. In the year ending January 31, 1971, 5,355 serological specimens were tested for an average of 8 viral antibodies per specimen. In addition, indirect tests (mouse antibody production) were performed on 94 specimens. The laboratory has the capability of performing serological tests detecting infection with 34 known viruses or closely related groups of viruses. Developmental studies on the detection of viral infections in cats have progressed to the field trial stages, with three cat colonies under surveillance. All serological procedures remain under evaluation with a spectrophotometric technique being developed to assist in reading complement titrations. This technique will increase the reproducibility of complement fixation tests.

The large spectrum of viruses encompassed in the virus detection work requires the production of a large and complex battery of viral reagents, control cells, and viral seed stocks. These materials are available to the scientific community on request through SVCP Resources. Research continues on the evaluation of the importance of various viral infections in laboratory animals to cancer research, both directly to cancer research work and in influencing the supply of healthy animals.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent and cat colonies and laboratory animal produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems. LCM virus, in addition to being infectious for humans, is difficult to detect. Significant contributions are being made to the knowledge of the natural history of several indigenous viruses of laboratory animals.

Proposed Course: Continue the rodent virus serodiagnostic service and develop a similar service for cat viruses. Improve the sensitivity and reliability of the tests. Apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

Current Annual Level: $400,000

MONTREAL CHILDREN'S HOSPITAL (PH43-65-1020)

Title: Procurement of Serum from Human Childhood Leukemia

Contractor's Project Director: Dr. Ronald L. Denton

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To obtain serum from a variety of pediatric oncology patients, family members, and controls for virologic study; to identify special cases for more extended workup.

Major Findings: The contractor has provided sera for a variety of SVCP investigators. The results of one serological study, using samples from Montreal Children's Hospital, were reported in a publication in J.N.C.I. One of these studies by Syracuse University investigators indicated that antibodies to avian leukosis virus were not present in sera from patients with acute leukemia. Two other studies, one involving other animal leukemia viruses and another involving Australia antigen, are still under way. Samples from a patient with probable progressive multifocal leukoencepholopathy were provided to Dr. George Todaro to determine whether the virus-like particles identified in similar cases could be isolated and characterized.

The contractor participated in a publication indicating that infectious mononucleosis was associated with a favorable clinical course when occurring in the course of acute leukemia.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract for the supply of serum and tumor specimens from pediatric oncology patients and suitable controls. An increasing need for tissues and blood samples from pediatric oncology patients requires a number of resource contracts to meet SVCP needs.

Proposed Course: Continue to collect serum specimens, especially from selected leukemia patients, for identification of host factors associated with long-term survival.

Date Contract Initiated: September 24, 1965

Current Annual Level: $24,540

NEW YORK STATE VETERINARY COLLEGE AT CORNELL UNIVERSITY (NIH 70-2224)

Title: Feline Tumor Viral Diagnostic Laboratory

Contractor's Project Director: Dr. James H. Gillespie

Project Officer (NCI): Dr. James T. Duff

Objectives: To complete production and evaluation of cat and dog viral reagents; to monitor cat cell cultures and other materials associated with cat tumors for indigenous cat viruses and other microorganisms.

Major Findings: The contractor has established a feline tumor viral diagnostic laboratory and has available all known indigenous viruses of the cat. The feline reagents (virus seed stock and antisera) include 16 viruses. The contractor has tested the final packaged reagents for purity, potency, homogenicity, and complete reciprocal neutralization. Although the packaged viruses have undergone a drop in titer of about two logs, these reagents are satisfactory for distribution to other investigators. Cross-neutralization tests are in progress. Two cat cell lines have been extensively screened for indigenous agents, and a limited amount of seed stock of these lines is being prepared. A serological test is being developed to detect cats carrying syncytial virus. Studies to determine the etiology of feline infectious peritonitis continue. The laboratory monitors viral tumor cell culture suspensions and other materials associated with cat tumors for indigenous feline viruses.

Significance to Biomedical Research and the Program of the Institute: An evaluation will be completed of reagents prepared for the feline viruses since these materials will be made available to NCI intramural and collaborating scientists as well as to the scientific community. The contract provides a central laboratory where materials isolated from neoplastic or normal cats can be sent to determine whether or not they contain indigenous feline agents, and/or for viral identification.

Proposed Course: (1) Initiation of a pathogenesis study of feline syncytial agent(s) in the domestic cat; (2) Monitoring of NCI-Cornell Specific Pathogen-Free Cat Colony for feline syncytial agent(s); (3) Study a feline syncytial agent and a feline adenolike virus *in vitro* for their oncogenicity "switch-on" effect on type C particles; and (4) Continue the operation of a feline virus diagnostic laboratory.

Date Contract Initiated: June 25, 1970

Current Annual Level: $35,295

UNIVERSITY OF PADUA (PH43-68-1389)

Title: Collection of Human Tissue Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To procure and ship human tumor and other tissue specimens to NCI for use in research programs.

Major Findings: Skin biopsies have been obtained from inbred and isolated populations in the Dolomite Mountains in Italy and have now been grown in tissue culture. Some specimens have shown a high virus-transformation susceptibility. Genealogical information on the donors is being analyzed to determine if the susceptibility to oncogenic transformation has a genetic correlation. These cell lines will be employed in a human oncogenic virus detection program organized by NCI.

Significance to Biomedical Research and the Program of the Institute: The skin biopsies will be used in a project to detect human oncogenic viruses *in vitro* and to determine the significance of the transformation test to oncogenesis.

Proposed Course: To collect and culture human skin biopsies as previously, along with genealogies.

Date Contract Initiated: October 27, 1964

Current Annual Level: $7,938

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH 69-93)

Title: The Production of Simian Viruses and Homologous Antisera

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. James T. Duff

Objectives: To determine the quality of simian virus reference reagents (seed material and antisera) packaged for NCI by another contractor. In addition, the laboratory serves as a diagnostic laboratory in a limited capacity for viral isolates that may emerge from studies done by other SVCP contractors.

Major Findings: The majority of the packaged simian virus reference reagents have been tested. The laboratory has assisted NCI in the identification or differentiation of the Mason-Pfizer mammary virus, *Herpesvirus saimiri*, a wooly monkey virus, and other unknown primate viruses isolated from neoplastic tissues.

Significance to Biomedical Research and the Program of the Institute: These reagents will be useful to investigators in characterizing viruses isolated from neoplastic diseases (natural or induced) that occur in primates, and for monitoring primate colonies.

Proposed Course: Certify the packaged simian virus reagents and serve as a diagnostic laboratory for viral isolates referred to SWFRE by NCI.

Date Contract Initiated: June 15, 1966

Current Annual Level: $5,775

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH 69-2011)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. Roy F. Kinard

Objectives: To supply young chimpanzees to SVCP investigators.

Major Findings: Four infants were born this year. Two were sent to Dr. J. Melnick and two await shipment until he has suitable inocula. No abortions or stillbirths occurred this year. All six proven females are pregnant or being rebred.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. This is the only source of newborn chimpanzees for SVCP.

Proposed Course: Continuation with no significant change.

Date Contract Initiated: April 25, 1969

Current Annual Level: $24,961

UNIVERSITY LABORATORIES, INC. (PH43-66-1133)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officer (NCI): Dr. Robert Holdenried

Objectives: Production of leukemia and sarcoma viruses and antisera.

Major Findings: Production of murine sarcoma virus (Moloney), partially purified from BALB/c mouse tumors, proceeded through the year without complication. The amount now in storage appears sufficient for a couple of years' anticipated research requirements. Production of increased yields of the IC strain of Moloney leukemia virus from mouse spleens is partially replacing the sarcoma virus production. The leukemia virus, as a 10% cell free extract from the spleens, has an unusually high titer of 7-8 logs of focus forming units per ml. Antisera to the Moloney sarcoma virus (Moloney) has been produced in mice by inoculation of increasingly concentrated virus over an 8-month period. Sera from these mice have a high neutralizing titer. Mouse antisera to Moloney leukemia virus is under preparation. About eight liters of RSV (RAV-1) have been grown in tissue culture. Between three and four liters of RAV-1 have been produced.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera has been extensively used by the SVCP and the research community.

Proposed Course: The contractor is in the process of converting to large scale cell culture propagation of oncogenic viruses. Propagation of cat leukemia virus should commence by April 1971, and Schmidt-Ruppin RSV should be underway by July 1971. Other virus production will be adjusted to meet SVCP requirements.

Date Contract Initiated: June 4, 1962

Current Annual Level: $238,000

ZOOLOGICAL SOCIETY OF SAN DIEGO (PH43-63-56)

Title: Experimental Breeding of Small Primate Species

Contractor's Project Director: Dr. Robert W. Cooper

Project Officer(s) (NCI): Drs. Roy F. Kinard and Robert Holdenried

Objectives: To breed several selected small primate species and supply offspring to SVCP investigators; to develop antilymphocyte sera for these species; to develop and use tissue cultures in selected in vitro virus experiments or assays.

Major Findings: Newborn animals were supplied to Drs. F. Deinhardt, Melendez, Valerio, John Moore (NIEHS), and A. Hendrickx (National Primate Center, Davis, California). Antithymocyte globulins (squirrel monkey and talapoin) were prepared and sent to Dr. Deinhardt. Extract of woolly monkey fibrosarcoma was also sent to Dr. Deinhardt, and this extract was inoculated into six squirrel monkeys, causing two nodules. A cell line from a squirrel monkey tumor induced by FSV was developed and is available. Malignant sarcomas occurred in talapoins inoculated with Rous virus and squirrel monkeys with Rous and FSV. Six galagoes inoculated intratracheally with benzpyrene developed bronchogenic carcinoma.

Significance to Biomedical Research and the Program of the Institute: These small primate species are cheaper, easier, and safer to use than rhesus, baboons and chimpanzees, and at least squirrels and talapoins have been shown susceptible to virus cancer.

Proposed Course: Because of certain EEO requirements, this contract will be discontinued. The work may be continued at another location if contractual details can be worked out soon.

Date Contract Initiated: August 8, 1962

Current Annual Level: $100,000

SPECIAL ANIMAL LEUKEMIA ECOLOGY SEGMENT

Dr. Michael A. Chirigos, VBB, Etiology Area, Chairman
Dr. John P. Glynn, Chemotherapy Area, Vice-Chairman
Dr. George J. Burton, VBB, Etiology Area, Executive Secretary

CORNELL UNIVERSITY (PH43-65-620), ITHACA, NEW YORK

Title: Leukemia Studies in the Cat

Contractor's Project Director: Dr. Charles Rickard

Project Officer (NCI); Dr. John P. Glynn

Objectives: Cats with spontaneous leukemias and sarcomas will be subjected to virus isolation procedures, in an attempt to obtain C-type virus strains from a broader spectrum of neoplastic diseases in the cat. Oncogenic virus strains now available for research were derived from lymphocytic leukemia/ lymphoma or fibrosarcoma. Viruses from other leukemias and sarcomas are sought. The incidence of spontaneous leukemia and sarcoma virus infections in various populations of cats will be investigated by virus isolation in tissue culture, followed by demonstration of complement-fixing group-specific antigen (COCAL test), electron microscopy, demonstration of "helper" activity or interference in focus-forming tissue culture procedures, and kitten inoculation.

Cats which have been in contact with human cases of leukemia or sarcoma will be examined for presence of cat leukemia or sarcoma viruses. A virus isolated from such a cat can be used in serological tests of sera from the associated human patient and his family, to investigate the possibility that cat leukemia or sarcoma viruses have infected humans. The sites of replication of cat leukemia and sarcoma viruses, and their possible mechanisms of horizontal transmission will be investigated. Insect transmission will be attempted. The infectivity of various cat leukemia and sarcoma viruses will be tested in cats, dogs, and tissue cultures derived from various animal species.

Major Findings: (1) A direct fluorescent antibody (FA) test has been developed for the demonstration of the group-specific antigens of feline leukemia and sarcoma viruses. This is a sensitive method for the detection of infected cells from animals or tissue cultures. It provides a means of isolation of feline leukemia and sarcoma viruses by inoculation of tissue cultures, enhancement of viral concentrations by propagation for 2-3 weeks, and application of the fluorescent antibody test. The same method permits titration of the viruses by inoculation of tissue cultures with serial 10-fold dilutions, growth for 10-20 days, and examination of the cultural cells by the FA technique. Cat embryo cells were the most susceptible to infection by minimal doses (10^{-7}) of feline leukemia virus, dog embryo cells somewhat less (10^{-6}), and human embryo cells still less susceptible (10^{-4}), in a comparative study. The FA test was significantly more sensitive and discriminating in the demonstration of feline leukemia and sarcoma infections than the agar gel immunodiffusion, complement fixation, and electron microscopic techniques. It was used effectively in serum neutralization tests.

(2) Feline leukemia virus was demonstrated in 20 of 25 cats with lymphoma/lymphocytic leukemia; 3 of 4 cats with myeloproliferative disease; and 1 of 4 cats with mast cell neoplasia. Virus was not found in single cases of spontaneous feline osteosarcoma and liposarcoma. Budding C-type virus resembling feline leukemia virus was found in 1 of 2 mammary adenocarcinomas; its oncogenic activity was not determined. No virus was demonstrated in 15 animals which did not have a history of association with other cats with leukemia; however, leukemia virus was found in 3 of 6 clinically normal animals from a high leukemia incidence cat colony, and in 5 of 16 contact controls in experimental litters in which other kittens had been inoculated as newborns with feline leukemia virus.

(3) Serums from 17 cats with spontaneous lymphoma, myeloproliferative disease, or mastocytoma were tested for antibodies in agar gel immunodiffusion reactions against antigens from our strain of leukemia virus and the Gardner strain of sarcoma virus (with its "helper"). Six sera gave a precipitin line against the leukemia virus, 4 against the sarcoma virus, and 2 against both viruses. A study is under way to determine more specifically which antigens these spontaneous cat antibodies were directed against. The sera from 3 non-leukemic cats which gave agar gel precipitin lines against the sarcoma virus also had neutralizing activity against the same virus, suggesting that the agar gel tests detected antibody against viral coat antigens.

(4) Of 73 newborn or fetal dogs inoculated with feline leukemia or sarcoma viruses, 27 developed lesions of leukemia or sarcoma. The FSV-induced sarcomas tended to regress; dogs carrying tumors had undetectable or low antibody titers against the feline group-specific antigens. Feline leukemia virus induced lymphocytic leukemia/lymphoma in 10 dogs of 17 inoculated in 3 litters, representing 3 successive cell-free passages. The lesions were disseminated lymphosarcomas involving the thymus gland, various body lymph nodes, and other lymphatic tissues. C-type virus was readily demonstrated by E.M. in the neoplastic tissues. The incubation periods averaged about 60 days. This experimental lymphocytic leukemia of the dog is of sufficiently high incidence and short latent period that it should be useful for chemotherapy and immunotherapy studies. Some of the dogs inoculated with feline leukemia virus had serum antibodies demonstrable in the agar gel precipitin test, but the titers and identification of the antibodies has not yet been determined.

(5) A feline liposarcoma virus has apparently been demonstrated in a tissue culture inoculated with our strain of feline leukemia virus. It had been reported earlier (J.N.C.I., 42:987-1014, 1969) that liposarcomas occurred in experimental kittens in the first and second passages, but not in later passages. In the original and later experiments, 6 of 60 kittens in the first and second passages developed liposarcomas. In the present work, cell-free virus from the neoplastic thymus gland of the original spontaneous case (cat F-161) was inoculated into a cat embryo cell tissue culture. After 25 tissue culture passages, the cells transformed (whereas uninoculated

cultures did not). Cell-free tissue culture fluid from the transformed culture, when inoculated into newborn kittens, produced multiple liposarcomas, lymphosarcoma, or both. It would appear that both liposarcoma and lymphocytic leukemia viruses are present in the transformed tissue cultures.

(6) The group-specific internal antigens of feline leukemia and sarcoma viruses have been studied, in part with the collaboration of Professor Werner Schäfer of the Max-Planck Institute for Virus Research in Tübingen, Germany. One component with a molecular weight of about 15,000 was found only in feline leukemia and sarcoma viruses, was considered specific for the feline species, and was referred to as feline "gs.-spec. antigen". Another component with a molecular weight of about 33,000 was found to be shared by leukemia viruses of other mammalian (but not avian) species, and was called the "gs.-interspec. antigen". Evidence was obtained for the presence of the gs.-interspec. antigen in the 1.16 density gradient band of tissue culture fluid from a culture of bovine lymphosarcoma cells, and from two human neoplasms--the Levine III tissue culture from a case of mammary carcinoma and 6410 from a case of chronic myelogenous leukemia.

(7) Feline leukemia or sarcoma viruses, or infected cells, have been supplied to 18 other research workers. There have been 13 publications.

Significance to Biomedical Research and the Program of the Institute:
The close association of cats with humans suggests that the possible common etiology of human and cat leukemia and sarcoma be carefully investigated. In addition, cat cancer studies will provide the most useful and practical model systems for research. It appears that cat leukemia virus can under certain circumstances, cross species barriers to induce tumors in the dog, which is also closely associated with man.

Proposed Course:
(1) The incidence of feline leukemia and sarcoma viruses will be determined in various cat populations, using isolation in tissue culture and fluorescent antibody testing of these cultures.

(2) Serological tests for antibody in cat sera will be investigated further.

(3) Attempts will be made to demonstrate the "gs.-interspec." antigen in leukemia and sarcoma viruses, infected cells, and other suspect materials from various animal and human neoplasms. Further refinement of reagents for these tests will be carried out.

(4) Field isolates of C-type viruses from feline lymphocytic leukemias, myeloproliferative diseases, mast cell tumors, various sarcomas, and other sources, will be compared in neutralization tests in tissue culture for evidence of distinctive serotypes.

(5) Experimental kittens will be used to establish the viral etiology of additional feline leukemias, sarcomas, and other neoplasms. Newborn and fetal dogs will be inoculated with feline viruses to determine oncogenicity and antigenicity in the dog.

Date Contract Initiated: June 23, 1965

Current Contract Level: $315,000

RESEARCH FOUNDATION OF THE STATE UNIVERSITY OF NEW YORK, SYRACUSE, N. Y. (PH43-65-605)

Title: Comparative Studies on the Chemical and Physical Nature of the Known Leukemia Viruses and their Relationship to Particles Recovered from Other Sources.

Contractor's Project Director: Dr. Charles Burger

Project Officer (NCI): Dr. John P. Glynn

Objectives: To determine the physical and chemical nature of virus-like particles recovered from leukemic human and animal material and to determine the relationship of these particles to viral particles recovered from other sources. To collaborate with other SALES contractors in projects relevant to these objectives.

Major Findings: A total of 260 liters of tissue culture supernate from which FeLV is purified have been processed. Virus has been supplied to Cornell, NCI, and also sent to Tubingen and Chicago. FeLV has been characterized as composed of a single stranded RNA which has a buoyant density of 1.64 gm/cc (CsCl) and a M.W. of $\sim 2 \times 10^6$. Whole virus buoyant density in CsCl is 1.196 gm/cc. Integrity of isolated nucleoids depends upon presence of divalent cations (Mg^{++} or Ca^{++} at 0.01 M). Two isopycnic bands of nucleoid material are found upon precipitation of this material in the absence of divalent cations.

Isolated peptides of FeLV consist of three major units at M.W. of 12,000, 23,000, and 44,000. The 23,000 M.W. material is gs antigen. Other minor components are present. Radioactive nuclides (gallium 65, barium and indium) are more rapidly taken up by virally transformed tissue culture cells than by non-transformed cells. Negative results were obtained in testing human tumors for presence of helper activity using Moloney sarcoma defective virus.

Significance to Biomedical Research and the Program of the Institute: The major emphasis in virus separation studies involves human and animal materials within the Special Virus Cancer Program. Developmental research is conducted on separative procedures used for isolation, purification and concentration of candidate human and other animal agents. In addition to physical and chemical characterization studies, purified materials are also supplied to other investigators within the SVCP.

Proposed Course: This contract was terminated on September 30, 1970.

Date Contract Initiated: April 21, 1965

Current Contract Level: $44,405 for six months to June 30, 1970, and extended without funds to September 30, 1970.

UNIVERSITY OF CALIFORNIA (NIH-70-2048), DAVIS, CALIFORNIA

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Leo K. Bustad

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: (1) To determine the oncogenicity, comparative pathogenicity and the *in vitro* species infectivity of subhuman primate leukemia and sarcoma viruses; (2) continue studies to compare, *in vitro*, the species infectivity spectrum of feline sarcoma and feline sarcoma-leukemia virus complex in cell lines of various species; (3) determine the pathogenesis of the diseases induced by the sarcoma virus and the sarcoma-leukemia virus complex in different species, clinically and pathologically; (4) continue studies on the etiology and transmissibility of the feline myeloproliferative syndrome and initiate these studies on spontaneous and radiation-induced osteogenic sarcoma; and (5) perform cooperative studies on the radiation-induced myeloproliferative syndrome in dogs.

Major Findings:
Extracts of a spontaneous fibrosarcoma in a woolly monkey were used to infect cultures of normal woolly monkey muscle cells. C-type virus was isolated in an early passage and is being replicated. Electron microscopic examination of the cultures after the third and sixth passages showed a number of budding and mature virions measuring approximately 95 mμ. Maturation stages and buoyant density very closely resemble those of cat virus, size being the major difference. A suspension culture of lymphocytes from a gibbon ape with spontaneous lymphosarcoma is in the 14th passage. Electron microscopic examination has shown large numbers of extracellular particles morphologically similar to mature C-type oncogenic virions. The particles are approximately 95 mμ in diameter with an electron dense nucleoid of 65 mμ. Budding forms of the particle have not been detected. The particles come to rest in sucrose solution at a density of 1.14-1.16 g/cm^3. Immunodiffusion tests with rabbit anti-feline-leukemia virus-gs_1-antigen serum showed no reaction with the simian agents. Fluorescent antibody tests using dog anti-feline-sarcoma-serum, on cells replicating the simian virus, showed a positive reaction only with the gibbon ape lymphoid cells. Tests for RNA-dependent DNA polymerase showed positive reaction in woolly monkey virus as with AMV and FeLV. Species infectivity studies with woolly monkey virus showed positive infection in two human and two nonhuman primate cultures, but virus was not observed in bovine, feline or canine cultures. Species infectivity with gibbon ape virus is under way.

On six additional spontaneous feline fibrosarcomas examined by electron microscopy, two contained C-type viruses. Cell-free tumor material prepared from three of the six cats induced tumors in 17 of 20 kittens inoculated. Electron microscopic examination showed the presence of C-type budding viruses. Kittens given multiple subcutaneous inoculations had tumors whose size was related to the total quantity of inoculum administered. Studies on cell mediated immunity in cats with regressing tumors are under way, as are *in vitro* studies on the isolation of defective FSV in cells of several species. Efforts are being continued to establish a possible viral etiology in canine leukemia.

In in vitro infectivity studies, FSV has infected cultures from cat, dog, cow, monkey, and man. All but the human and monkey cultures produced infectious virions which could readily transform cat cells even after 10-20 passages. The virions from infected human cultures, detectable by electron microscopy, did not cause morphologic alterations of feline cultures after several passages. However, cat cells co-cultivated with infected human cells did cause morphologic alteration of feline cultures after several passages.

Clones of FSV-infected, transformed bovine and feline cultures have been isolated and will be assayed for possession of a defective FSV genome that could be used to determine or rescue a leukemogenic agent from spontaneous cases. Relative to the effects of dose and host age on the pathogenesis of feline fibrosarcomas, it was found that decreasing the dose had similar effects to increasing the age. Characteristics observed were a progressive increase in the latent period, an increase in the percentage of tumor regressions, a decrease in the maximum tumor size attained, a decrease in metastatic potential, histologic changes in tumor morphology, and a decrease in the ease of locating viral particle by electron microscopy. An apparent increase was noted in resistance to tumors by kittens raised on queens previously used to raise FSV- or FeLV-inoculated kittens. Attempts to produce FSV in sheep were successful only in sheep less than 60 days of fetal age, which had not yet acquired immune competence. The three tumors so produced all regressed within 30 days.

C-type virus identical to feline leukemia virus was found in association with bone marrow cells in a variety of feline myeloproliferative diseases. Canine lymphosarcoma and myelogenous leukemia were transplanted by in utero inoculation of fetal puppies. Whole cell suspensions from tumors were used to transmit lymphosarcoma. Gross and histopathologic evaluations on first and second passage lymphosarcoma recipients are complete. Chromosome determinations show cellualar invasion of all recipients' organs by donor cells, and the emergence of a hypodiploid cell line. Preliminary electron microscopic examination of tissue from pups with myelogenous leukemia has not shown any virus-like particles.

In the bovine study, about 12 liters of plasma was collected and concentrated by continuous flow-zonal untracentrifugation for attempts to determine whether virus-like particles isolated from inoculated cattle can be considered as a leukemogenic agent.

Significance to Biomedical Research and the Program of the Institute

The proposed research objectives are relevant to human leukemia in that they will enable determination of whether the oncogenic animal viruses under study can infect and transform cells of different species, including man, or induce neoplastic changes in selected animal species. The suspected viral etiology of two subhuman primate tumors are of special significance because of their obvious close relationship to man.

The popularity of cats and dogs as pets has increased tremendously and their importance in viral oncology as models and vectors is still of great interest and was reviewed in last year's proposal. The human population is in close and constant association with dogs and cats, which are both known to be carriers of communicable diseases. Of particular concern too is that children have the most intimate association with these pets. The use of milk from leukemic dairy cattle as a possible hazard to man has been reviewed frequently. Whether bovine, feline, and canine neoplastic diseases are communicable to man is a question that must be determined. It is possible that the cat is a reservoir for leukemia viruses, and that leukemia in man may result from incidental infection. The fact the the group-specific antigen of FeLV is also common to other animal leukemias suggests that the cat leukemia virus may be the prototype leukemia virus for all leukemias, including those of man. Although the evidence is incomplete, it enables speculation that cats may serve as excellent models for a full understanding of lymphoreticular neoplasia, sarcomas, and myeloproliferative disorders of man. The radiation-induced myelogenous leukemia in dogs now under study appears to be the best model for the study of this disease in man.

Future Course:

Characterization and species infectivity studies of the woolly monkey fibrosarcoma and gibbon ape lymphosarcoma agents will be continued. These studies will include biochemical, biophysical, and serological characterization, as well as biological activity of the virus *in vitro* and *in vivo*. The development of assay methods for the detection of oncogenic simian virus will be continued. Investigations will continue on clone isolation and characterization of FSV-transformed heterospecies cells of various species. The investigation of the pathogenesis of FSV tumor induction and regression will be continued. The search for an assay for detection of immune response to FSV-induced tumors in cats will be continued. Studies on spontaneous tumors in canine, simian and other animal species will be continued for the establishment of the viral etiology.

Date Contract Initiated: November 16, 1969.

Current Contract Level: $456,192.

HAZLETON LABORATORIES, INC. (NIH-69-2079), FALLS CHURCH, VIRGINIA

Title: Etiology of Cancer in Dogs

Contract's Project Director: Dr. Erling M. Jensen

Project Officer (NCI): Dr. Wilna Woods

Objectives: The overall objective of this contract is to determine whether any of the cancers of dogs are caused by a virus(es) and whether there is any etiological relationship between canine cancer and human cancer. Among specific objectives are cellular and cell-free transmission of canine lymphomas and sarcomas; histopathologic, immunologic, serologic, hematologic and virologic, tissue culture and electron microscopic studies on spontaneous and induced canine neoplasias, as well as studies to determine whether a canine leukemia/sarcoma complex exists in the canine system.

Major Findings: Whereas emphasis previously had been placed on malignant lymphomas, the program has now been broadened to investigate the etiology of all cancers in dogs. Seventeen different tumor tissues have been acquired, including mammary tumors, perianal gland adenocarcinomas, osteosarcomas, fibrosarcomas and mast cell tumors. Cultures of many of these have been initiated for in vitro studies and serum specimens have been collected for serological studies.

In view of the lack of success in finding an oncogenic virus by direct methods such as electron microscopy, animal inoculation, tissue culture inoculation and in vivo and in vitro tumor passage, emphasis has now been placed on indirect methods of detecting virus such as the search for viral antigens by immunological means and virus associated enzymes by biochemical techniques. In preparation for these studies, antisera against murine and feline leukemia viruses prepared in goats, rabbits and guinea pigs have been obtained and tested against intact and extracted murine and feline virus preparations, as well as mouse cells infected with murine viruses, and cat and dog cells infected with feline leukemia and sarcoma viruses. Specifically, complement fixation, immunodiffusion and indirect immunofluorescence tests are being employed, and all of these test systems have now been perfected. A serum lot from goats immunized with feline leukemia virus has been found, which after appropriate absorption, reacts specifically in the immunodiffusion and immunofluorescence tests with the interspecies group antigen (g.s. 3) common to murine and feline leukemia viruses. This serum, however, is anticomplementary, and new lots of serum must be obtained and evaluated for use in studies employing the CF test. Immunofluorescence studies are now being initiated to survey the tumor cell cultures mentioned above, and others as they become available, for the presence of this group antigen.

The combined effectiveness of chemotherapy and immunotherapy on canine lymphomas is being investigated. Previous reports from this laboratory have described a transplantable lymphosarcoma-mast cell tumor which has now been through 11 passages in newborn dogs. A tissue culture line derived from this tumor and transplantable in dogs will be very useful in in vivo therapy studies, since it affords a well characterized and reproducible tumor system. Experiments have been conducted to determine the optimal inoculum dose and age of inoculation for such studies. Results to date show that tumor induction requires at least 1×10^6 cells administered intraperitoneally or 1×10^5 cells intramuscularly, and that tumors do not develop when inoculated later than 10 days post partum.

Since combined chemotherapy and immunotherapy studies are also anticipated in dogs bearing spontaneous tumors, eight lymphosarcomatous dogs have been obtained, and five of these have been used in dose-response studies with cyclophosphamide. The three remaining dogs are being evaluated to determine the course of their disease prior to administering therapy. Once the optimal regimen of cyclophosphamide therapy is established to gain remission of the tumors, immunotherapy will be initiated.

Preliminary studies to determine the effect of chemotherapeutic agents on the immune response in normal dogs have been conducted to assess whether these agents will interfere with subsequent immunotherapeutic attempts. Results thus far show that a single dose of 20 mg/kg of cyclophosphamide, administered either before or after antigenic stimulation, causes a marked decrease in the total peripheral leukocyte count but does not affect either the primary or secondary humoral immune response. Studies are now in progress to evaluate the drug effect on the cellular immune response. Specifically, it will be determined whether the compound affects the ability of dogs to become sensitized to dinitrochlorobenzene.

Significance to Biomedical Research and the Program of the Institute:

Because of the close relationship of dogs to man, as an intimate member of his household, it is important to determine whether canine tumors are induced by viruses, and whether such viruses can infect other species, including man. By isolating, identifying, and studying viruses found in both canine and human leukemia, lymphomas, and sarcomas, similarities may emerge which may link the disease in dogs with that of man. As a corrolary, it is also necessary to determine whether oncogenic viruses of cats can pass to man through dogs.

Proposed Course:

1. A significant number of new tumor specimens and sera will be received during the next report period. Attempts will be made to culture as many of these as is feasible and each culture will be tested by immunofluorescence against the group specific antiserum as well as the tumor autologous serum and selected sera from tumor-bearing dogs. In support of these studies, selected sera from tumor-bearing dogs will be tested by the complement fixation technique against known murine and feline group antigens. Transplantable tumors currently available in their laboratories will also be examined thoroughly.

2. Attempts to recover an occult virus by established virus rescue techniques have been conducted in the past to a limited extent and these will be pursued further. The techniques include superinfecting dog tumor cell lines with feline and murine leukemia viruses, cocultivation with leukemia virus-infected cells, and fusion of dog tumor cells with normal and leukemia virus-infected cells using inactivated Sendai virus. During the next report period the methodology will be developed using known murine systems, and studies will be initiated employing some of the most promising canine systems currently available in their laboratories.

3. Arrangements have been made with Dr. Robert Gallo of NCI for the preliminary testing of one or two canine tumors for the presence of an RNA-dependent DNA polymerase. Should these tests prove promising, arrangements will be made for the further testing of a variety of canine tumors for this enzyme. Assuming that the enzyme is a product of a virus genome, tumors and tissues can be selected which are the most promising candidates for virus rescue studies.

4. Mast cell tumors have been reported twice in the literature to be

transmissible by cell-free extracts. In collaboration with Dr. Charles Rickard of Cornell University, arrangements have been made to obtain one of these strains for study in Hazleton Laboratories. Intensive efforts will be made to demonstrate the presence of a virus in this system by rescue and immunological methods already described, as well as other *in vitro* and *in vivo* methods, such as *in utero* inoculation of cells and cell-free extracts, tritiated uridine uptake studies and electron microscopy.

Date Contract Initiated: May 26, 1969

Current Contract Level: $288,000 for 6 months.

UNIVERSITY OF PENNSYLVANIA (PH43-65-1013) NEW BOLTON, PENNSYLVANIA

Title: Research in Experimental and Natural Transmission of Bovine Leukemia

Contractor's Project Director: Dr. Robert Marshak

Project Officer (NCI): Dr. John P. Glynn

Objectives: To determine the etiology of bovine leukemia. To attempt experimental transmission of bovine leukemia through use of cell-free materials prepared from selected leukemic milk and solid tissue. To investigate natural transmission of the disease through reciprocal foster nursing experiments.

Major Findings: Three experiments (differential centrifugation + sucrose density gradient centrifugation (SDGC); polyethleneglycol precipitation + SDGC; and rate zonal centrifugation + SDGC) were conducted in an attempt to isolate, concentrate and purify the VLP in cell line NBC-6. In all three experiments, band three (3-5 bands were present in each preparation) contained occasional C-type VLP. Thus far, none of the procedures is deemed suitable for producing highly concentrated virus preparations, possibly because VLP appears to occur in large aggregates in the cell cultures. The presence of budding particles in PHA treated and untreated buffy coat (BC) cells from leukemic cow BF044 is now routinely demonstrated. C-type particles were present in BC cells from 4/7 cows in their multiple-case herd and in 0/5 cows in a leukemic-free herd. Identical budding C-type particles have been shown to be present in 3 different leukemic bovine cell lines.

Helper Assay Studies: Using the *in vivo* system described by Steeves (JNCI 44: 587, 1970), they showed helper activity in five of six extracts from PHA-treated BC cultures from BF044. Two untreated cultures have given questionable results and helper activity was also present in four extracts of BC cultures from a normal cow (2 PHA-treated and two untreated.). The greatest helper activity was observed in a cell-free extract prepared from NBC-6 cells cultured 48 hours in E20FCI. Such cultures contain replicating C-type virus. The presence of helper activity in the presumably virus-free preparations remains to be explained. In cooperative studies with Dr. Bassin (NIH), attempts to demonstrate helper activity by candidate bovine materials in the MSV system were unsuccessful.

Immunological Studies: Cell line NBC-6 was examined by indirect immunofluorescence using acetone-fixed cell preparations. In the first series of experiments, cells showing a granular and/or diffuse brilliant fluorescence in the cytoplasm were consistently found when tested with the serum from a regression case of bovine leukemia (cow #27-125). The percentage of fluorescent cells was significantly higher in the cultures maintained in E20FSI than in the cultures maintained in M20HSI. Subsequent EM and IF tests, performed in the same cultures of NBC-6 cells grown in E20FSI or in M20HSI, confirmed the close correlation between cells showing VLP and fluorescent cells. Sera of normal cow (BI 205) and of a normal calf from a leukemia-free herd were negative. Preincubation of fixed NBC cells with the serum or 6-globulin fraction of the regression case (27-125) completely blocked the fluorescence activity of FITC conjugated y-globulin from 27-125. No blocking activity was found in the serum or y-globulin from normal cow BI-205. A preliminary serological survey showed the presence of fluorescent antibodies to NBC-6 cells in the sera of 11 out of 13 leukemic animals (12 cows and one calf). Ten additional sera from normal cows in a leukemia-free herd were tested and none showed IF antibodies.

Studies on Bovine Syncytial Virus (J-BSV) - On the basis of 1,452 sera (from 24 herds) which have been studied to date, the following limited conclusions are drawn: a. The distribution of J-BSV infection varies greatly from herd to herd regardless of the herd's leukemia status; b. There appears to be a significantly greater rate of infection in multiple case herds when compared to contact herds, and both of these show significantly higher rates than the control herds tested so far; as yet, no relationship between J-BSV infection and persistent lymphocytosis is apparent.

Reciprocal Foster Nursing Experiment - Oldest animals are 37 months old; youngest are 14 months. No leukemias have been detected so far. Though too early for definitive analysis of the lymphocytosis status of the various categories of animals, as yet there have been no alterations in absolute lymphocyte counts which can be related to the types of foster dams upon which the calves nursed.

Studies on the Experimental Production of Leukemia - To date, no clinically detectable leukemias have developed, but collectively the cattle in the gross filtrate, leukemic suspension culture cell line (CL) and the leukemic thoracic duct lymph (TDL) transmission experiments have all shown significantly elevated absolute lymphocyte counts (ALC). A significant drop in the ALC of four out of five cattle in the leukemic cell line experiment may be significant because, in spontaneous cases, such a drop in ALC commonly precedes the appearance of tumor masses. Chromosome studies in several animals which received i.v. infusions of CL and TDL also suggest transmission of the disease. A new transmission experiment in which colostrum-deprived neonatal calves are receiving NBC-6 suspension culture cell grown in Eagle's medium with 20% FCS (such cells are rich in budding C-type virus) is underway.

Significance to Biomedical Research and the Program of the Institute: Since there is a close association between cattle and their products, and man, the etiology of bovine leukemia is of utmost importance. This program has been making progress, and there has been active collaboration with many other investigators.

Proposed Course: 1. Virus isolation and concentration experiments will be extended. 2. An investigation of the presence of RNA-dependent DNA polymerase will be conducted using virus preparations obtained from NBC cell lines and from short term BF044 BC cultures. The presence of the enzyme in cells from previous cultures as well as in lymphocytes from leukemic and non-leukemic cattle will be determined. 3. Search for the gs antigens of leukemia viruses in preparations from NBC cell lines and BC PHA treated cultures. 4. Attempt to transmit bovine C-type viruses to bovine embryo fibroblasts, NBC-10 cell lines to other established cell lines. 5. Attempt to develop an in vitro infectivity assay for the bovine C-type viruses.

Date Contract Initiated: June 18, 1965

Current Contract Level: $676,126

FLOW LABORATORIES, INC., ROCKVILLE, MARYLAND (PH43-67-1396)

Title: Immunological Studies of Mammalian C-type Viruses.

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officers (NCI): Dr. Michael Chirigos
Dr. Robert J. Huebner

Objectives:
(1) To determine the relationship between the envelope antigens of authentic (indigenous) hamster C-type viruses (D-9, Graffi) and the hamster pseudotypes. To prepare antibody for each virus and carry out reciprocal neutralization tests where possible. To carry out serum blocking tests (e.g., does D-9 virus absorb antibody to M-MSV(HaLV)?) and virolysis tests will be performed. (2) To investigate the occurrence of hamster virion antigens (mainly the group-specific antigen) in normal hamsters at various developmental stages (as has and is being done by Huebner and colleagues in the mouse). These studies will be carried out by complement-fixation and gel diffusion. (3) To analyze tumor-bearing dog serum (FeSV induced) for neutralizing and group-specific antibodies to the cat C-type viruses by a variety of methods. (4) To test in vivo activity of canine derived sarcoma and non-focus forming (leukemia?) virus in dogs, rats, mice and hamsters. (5) To test a number of dog tumors derived from feline sarcoma virus inoculation, but which now appear to be virus-free for presence of a defective sarcoma virus genome.

Major Findings: Non-transforming helper viruses were isolated from pools of two hamster-specific sarcoma viruses in which isolations by terminal dilution procedures had previously been unsuccessful. This presumably results from approximately equivalent titers of sarcoma and helper viruses in the original pools. Helper virus was isolated from morphologically normal cells selected from tissue culture plates showing relatively few transformed colonies, and by endpoint dilution from cloned transformed cells. Focus formation by the hamster-specific sarcoma viruses was independent of virus spread, and non-producer cell lines carrying the sarcoma virus genome were isolated; thus, these viruses are apparently unable to replicate independently of helper virus. Hamster-specific viral antigens were not detected in hamster cells infected productively or non-productively with a murine sarcoma virus; thus, evidence for virus activation in vitro by the sarcoma virus genome was not obtained.

A serological survey of adult and embryonic hamster tissues using guinea pig antiserum to the hamster C-type virus group-specific antigen was made. A preliminary assessment of tests carried out at NIH and Flow show a low incidence of reactivity with adult tissues (≈5%) while whole embryo pools and embryonic kidneys appeared almost uniformly reactive. Attempts to isolate infectious virus have been negative. Tissue culture lines and tumor extracts of polyoma, SV_{40} and adenovirus induced tumors were negative, as were a number of "spontaneously" transformed cell lines. In contrast, several cell lines from tumors produced originally with cells transformed by chemical treatment (3-methylcholanthrene and fractions of cigarette smoke) were positive and virus particles were detected. A single pre-inplantation cell line has given suggestive evidence of the presence of virus. The latter work has been carried out with Dr. A. Freeman, MAI. At present, the hamster appears to closely resemble low incidence strains of mice in that the viral genome is probably present in every animal but only expressed or detectable at certain developmental stages and after special conditions of induction.

Significance to Biomedical Research and the Program of the Institute: This contract has direct relevance to the total program on RNA C-type viruses. The principal investigator and his associates have in the past played critical roles in developing methods for virus/antigen detection in isolation, production and assay, and have made major contributions to defining the mechanisms of carcinogenesis in cell systems involved in their own and related study programs. The canine and hamster C-type virus experience is of particular relevance since their convert nature appears to closely approximate the human experience. Technological developments in isolating and characterizing the canine and avian C-type viruses/antigens will have direct applicability to the human problem.

Proposed Course: This contract is to be fused on February 1, 1971 with contract NIH-69-97, "Studies of Herpes viruses, Adenoviruses, and C-type Viruses in Relation to Oncogenic Potential." The following studies are planned: Extensive serological testing of a variety of hamster cell lines and tissues for gs antigen reactivity coupled with assays for virus particles indicated. Cross testing with other species C-type viruses in attempts to detect common antigens. Sensitive assays using antigens labeled with I^{125} will be developed for this purpose. Use of the DNA product of the virion polymerase to probe for homologous RNA or DNA sequences in various hamster cells.

Production of specific antibody in guinea pigs for the future gs antigens.

Date Contract Initiated: June 20, 1967

Current Contract Level: $185,000

HOWARD UNIVERSITY SCHOOL OF MEDICINE, WASHINGTON, D. C. (70-2178)

Title: Immunological Studies on Human Mammary Tumors and Other Neoplasms

Contractor's Project Director: Dr. Michael V. Viola

Project Officer (NCI): Dr. Paul Levine

Objectives:
(1) Survey of neoplastic breast cancer tissue culture lines for tumor antigens using fluorescent antibody and complement fixation techniques. Sera will be surveyed for tumor specific antibodies, also. This work will be done in collaboration with Dr. Robert Ting, Bionetics Research Laboratories. (2) Procurement of selected tumor specimens and establishment of neoplastic tissue culture lines from selected cancer patients, particularly those with leukemia, lymphoma and breast cancer. (3) Procurement of sera specimens from patients with neoplastic and non-neoplastic diseases and their families. Patients at Freedmen's Hospital as well as all inhibitants of Quitman County, Mississippi (population 23,000) will have sera collected and stored.

Major Findings: Specimens were procured and delivered to the following investigators at the NIH: (1) Dr. Harish Chopra: four breast carcinoma cell lines, lymphoma cell line, two rhabdomyosarcoma cell lines, one effusion from Burkitt tumor, one pancreatic carcinoma cell line, one lung carcinoma biopsy, one breast carcinoma biopsy, one Burkitt tumor biopsy, one effusion from breast carcinoma. (2) Dr. B. Eddy - three lung carcinoma biopsies. (3) Dr. E. Plata - two breast carcinoma biopsies. (4) Dr. R. Ting - 49 sera specimens from patients with breast carcinoma. (5) Dr. P. Levine - lymph node imprints from patient with Hodgkins Disease. (6) 250 sera specimens and clinical histories from patients with cancer.

Tumor specific immunity in humans with breast cancer and other malignancies was investigated using a mixed lymphocyte-tumor cell culture technique. Incorporation of tritiated thymidine into lymphocyte DNA was measured as an indication of cellular immunity to tumor antigens. Cells from neoplastic effusions were used as the main source of tumor cells. 18 patients have been studied; at present completed data have been obtained on the first six patients studied. Lymphocyte stimulation by tumor cells in excess of control cultures were obtained in the following patients:

Diagnosis	#pos. / # tested
lymphosarcoma	1 / 1
breast carcinoma	1 / 1
lung carcinoma	1 / 1
non-neoplastic effusions	0 / 3

An American patient with the clinical and histo-pathological characteristics of Burkitt's lymphoma was studied. No virus particles were found by electron microscopic examination of tumor specimen and neoplastic effusion. EB virus antibody titres ranged from 1:8 to 1:320. Indirect immunofluorescence using the patient's acetone-fixed tumor cells and high-titred anti-EB sera (from a patient with nasopharyngeal carcinoma) was negative; however, mixed lymphocyte-lymphosarcoma cell culture revealed stimulation of normal lymphocytes by neoplastic cells. Therefore, it appears that this American patient with Burkitt's tumor has tumor specific antigens, probably unassociated with the EB virus.

A number of tissue culture lines were established from tumor specimens. Three lines are most likely to represent true neoplastic cell lines: (1) Two breast carcinoma cell lines - derived from malignant pleural effusions. Both epitheliod monolayer lines grow in a distinct alveolar pattern. (2) One rhabdomyosarcoma monolayer cell line. (3) One lymphoblastoid cell line derived from lymph node of American patient with Burkitt's lymphoma.

Response of patients lymphocytes to isogeneic cell lines is presently being studied. Dr. Harish Chopra is in the process of screening these cell lines for virus-like particles.

Significance to Biomedical Research and the Program of the Institute:

In the light of recent observations of virus-like particles in specimens from breast tumor patients (e.g., pleural effusions), including C-type and smaller particles, and in view of the recent isolation of C-type particles in a spontaneous mammary tumor in a rhesus monkey, follow-up studies on mammary tumors in humans (and other species) currently represents a very high priority in the Special Virus Cancer Program.

Proposed Course: (1) Lymphocyte sensitivity to tumor antigens will be studied further using: (a) Additional patients with neoplastic and non-neoplastic effusions. (b) Trypsinized solid tumors and neoplastic cell lines. (c) Cell-free effusion fluid as a source of antigen. (2) In patients demonstrating positive in vitro lymphocyte response, membrane antigens will be isolated from tumor cells and stored in liquid nitrogen. This antigen source will be used for serial testing of patients throughout the course of their disease. (3) Transfer factor will be isolated from lymphocytes from patients with positive response to isogeneic tumor cells. Unresponsive lymphocytes from a patient with a tumor of the identical cell type will then be sensitized with dialyzable transfer factor from the responsive patient. Positive in vitro transfer to tumor specific immunity by transfer factor would indicate common tumor antigens in tumors of the same cell type, and would be the first step in the clinical evaluation of the usefulness of transfer factor. (4) Tumor specimens and sera from cancer patients will be collected for NIH and SVCP laboratories desiring specimens. (5) Tumor specimens and cell lines derived from tumors will be supplied to Dr. H. Chopra for electron microscopic screening.

Date Contract Initiated: April 27, 1970

Current Contract Level: $38,800

NATIONAL COMMUNICABLE DISEASE CENTER, ATLANTA, GEORGIA (NCI-VCL-(68)-42)

Title: Etiologic Studies of Leukemia and Related Diseases Occurring in Unusual Epidemiologic or Genetic Situations

Contractor's Project Director: Dr. Clark W. Heath

Project Officer (NCI): Dr. A. Gazdar

Objectives: This is a collaborative program of the National Communicable Disease Center (NCDC) and the National Cancer Institute (NCI) in which epidemiologic and virologic studies are conducted of leukemia and related illnesses (cancer, congenital defects) occurring in unusual epidemiologic or genetic circumstances: community case clusters, human-animal case associations and situations in which cases appear associated in families. In selected population areas, case surveillance programs are operated to furnish accurate data on current leukemia incidence patterns and to assist in the prompt detection of unusual epidemiologic or genetic events. Each unusual event is investigated in detail, and when appropriate, specimens are collected from patients, persons with associated illnesses, family members and possible case contacts. While such investigations are pursued with a wide range of etiologic possibilities in mind, they focus primarily on viral oncogenesis. Specimens are examined both in the contractor's laboratory and in that of collaborating contractors, including bone marrow, serum, buffy coat cells, and tissue biopsies.

a. Field Investigations: A total of 31 field studies were pursued, 9 being investigations begun originally in earlier years. Sixteen concerned case cluster situations, 6 cases associated with unusual animal contacts (4 with cats and 1 each with horses and cows), 4 multiple case families, 3 leukemia in identical or like-sexed twins, and 2 marital leukemia.

In connection with these field studies, serum specimens were collected from 600 individuals, and blood and/or marrow cells for culture from 23. Of the serum specimens, 202 were obtained from a random sample of a high school population in California as part of an investigation of an apparent cluster there of osteogenic sarcoma, and 146 from a random sample of two grade school populations in Middletown, Connecticut, as part of a study of a case cluster of childhood leukemia in that town.

A cluster of three cases of lymphoma among children was found in the small town of DuBois, Pennsylvania. All three were from one Catholic parish and the two most recent cases were in schoolmates during one school year at that parish's parochial school. Their onset coincided with a sharp outbreak of varicella that affected that school both sooner and more severely than other schools in the area. The association of cases in this town with a particular church/school population segment is similar to earlier observations made in Niles, Illinois, Middletown, Connecticut, and Kendall Park, New Jersey. Case reporting continued from seven areas in the United States (Connecticut, Rhode Island, Utah, metropolitan Atlanta, Houston, Kansas City, and Nassau

County, New York). An additional surveillance project was begun involving childhood leukemia and lymphoma in the state of Colorado.

b. Laboratory Studies: Facilities at CDC for culturing cell specimens and performing virologic and immunologic studies on these and related sera were developed, starting in August 1970. Since that time 50 specimens of blood or marrow cells have been processed (preparation and storage of acetone fixed slides, in vitro tissue culture of cell materials), 6 in connection with ongoing field investigations, 12 on cultures received from Pfizer and developed there in past years in connection with earlier investigations, and 32 from patients with leukemia seen at local medical centers in Atlanta and north Georgia. Of these, 31 cell lines have been established and continued, each being built up for freezing back, for preparation of further acetone-fixed slides and, in the near future, for virologic and immunologic testing (fluorescent antibody and cytotoxicity testing with patient/contact sera, electronmicroscopic screening, and immunologic screening against known oncogenic agents (EBV, etc.). Cytogenetic analyses are being made of each cell line being processed.

Earlier in the year 11 skin fibroblast cultures were developed from a large multiple-case cancer family in California. These have been examined cytogenetically and sent to the Melpar laboratories for transformation studies. An analysis was made in collaboration with Dr. Don Morton, NCI, of the distribution of antibody titers against human sarcoma antigen in the 202 sera obtained from a high school population in Santa Clara County, California. No patterns of titer distribution were found to account for the pair of cases of osteogenic sarcoma diagnosed during that same school year in two classmates at the school.

Significance to Biomedical Research and the Program of the Institute:

Since this contract deals with epidemiologic and genetic studies on cases of human leukemia, and attempts to ascertain and clarify the etiology of human leukemia through intensive investigations of selected cases, it is of direct and immediate relevance to the aims of the Special Virus Cancer Program.

Proposed Course: Investigations of selected leukemia cases or cases of potentially related diseases will continue to be pursued, and appropriate serologic specimens will be collected. In the NCDC Laboratory, serologic and immunologic tests will be continued on collected specimens with attention focused on: a) search for oncogenic virus activity and b) sero-epidemiologic studies to help interpret individual family, community, or animal-related case clusters.

Date Contract Initiated: July 1, 1967

Current Contract Level: $144,200

ALBERT EINSTEIN MEDICAL COLLEGE, BRONX, NEW YORK (PH43-65-612)

Title: Research on Immunological Factors in Susceptibility to Murine Leukemia Viruses

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. John Glynn

Objectives: To determine the role of genetic and immunological factors in susceptibility of certain animals to candidate murine and human leukemia viruses specifically, the following studies were planned: (1) Examination of a cross involving tail-kinks gene and Fv-2 gene to determine on which side of dilute in linkage group 11 Fv-2 is situated. (2) Further studies to determine whether Fv-1 and Fv-2 control separate viral components (SFFV and LLV) in the Friend virus complex. (3) An experiment designed to clarify the basis for the observed loss of FMR and certain H-2 complex antigens from spleen cells during the course of the Friend disease. (4) Further studies with the apparently new virus line isolated by passaging F-B virus through B10.D2 mice. (5) Studies on the immunological unresponsiveness of certain strains of mice to the H-2.2 and to the FMR antigens. (6) Investigation of the genetic basis of the differential hematopoietic response in BABL/c and C_3H mice to F-B virus.

Major Findings: (1) A conference-workshop on "Genetic Factors in the Friend Virus Disease" was held in Bethesda on 22 January 1971, bringing together a number of workers with results which bear upon the interaction of virus genome in determining the outcome of infection with murine leukemia viruses. A broad area of agreement exists concerning the property of N-, B-, or NB-tropism of the viruses and response to the virus *in vivo* and *in vitro* as a function of the host's genotype at the Fv-1 locus. It was recognized that Fv-2, on the other hand, appears to influence the host response mainly to the SFFV component of Friend virus, and probably not to the LLV component. (2) Dr. Lilly's contribution to the correlation of the NB system with the Fv-1 system consisted in showing that a) the FV strains used *in vivo* to define the Fv-1 system differ in the NB system *in vitro* in the manner predicted; b) the congenic mouse strains used to define the Fv-1 system *in vivo* respond to N- and B-tropic viruses *in vitro* in the manner predicted; and c) all available data on susceptibility of various inbred mouse strains to FV *in vivo* correlated perfectly with the results. (3) Comparison of the congenic BALB/c (H-2^d) and BALB.B (H-2^b) mouse strains with respect to the quantitative expression of FMR antigen on their spleen cells shows that the antigen appears at the same time after virus infection and to almost the same extent; however, thereafter the amount of FMR on BALB/c cells declines enormously; whereas the amount on BALB.B cells declines only slightly. (4) B10.BR (H-2^k) and C3H.OH (H-2^o) mice are both highly susceptible to GV leukemogenesis; whereas their F_1 hybrids are quite resistant. Analysis of the F_2 generation (N=194) of this cross implies that this phenomenon of genetic non-complementation is due to two independent genes, neither of which is associated with H-2; however, further analysis shows that H-2 type significantly modifies this response, but not in an all-or-none manner. This is relevant to the recent findings that HL-A type is significantly but not completely correlated with human leukemia occurrence. (5) Studies on skin tumorigenesis by vaccinia virus and methylcholanthrene (MC) in a number of inbred mouse strains, and also in various crosses of the BALB/c and AKR strains, indicate that MC painting may induce a) skin tumors or b) leukemia, but rarely both, and that, if it induces skin tumors, one might infer that no covert infection with leukemia virus is present. (6) Absorption of FV by bone marrow cells of different genotypes. Suspensions of femoral bone marrow cells from three strains of mice were incubated with aliquots of a preparation of F-S virus and the supernatants assayed for residual virus activity in DBA/2 mice. By comparison with the unabsorbed control virus preparation, bone marrow cells from BALB/c and C57BL mice did not absorb significant amounts of virus, but cells from C3H mice removed all detectable virus activity (i.e., a reduction of at least 100-fold, by comparison with the controls). (7) Computer analysis of cytotoxic assays: Considerable effort has gone into the attempt to establish a computer program for the analysis of data obtained in the titration of isoantisera by the Cr^{51}-labelling method. Initial observations using this system appear to indicate that the cytotoxic assay is perhaps a more reproducibly quantitative technique than previously considered to be. (8) Since F-B virus always induces polycythemia in BALB mice but frequently induces anemia in C3H mice, they have examined the hematocrits of mice of the (C3H X BALB) X C3H mice at various times after virus inoculation. There is a significant difference in the mean hematocrit values of homozygous $H-2^k/H-2^k$ mice and heterozygous $H-2^k/H-2^d$ mice of this population which develops only during the third week after infection, and at about 23 days. The kd mice show a mean value at least 10 points higher than the kk mice in each of two experiments. Thus H-2 type is a factor in the polycythemic response to FV, and this should prove useful in analyzing the mechanism of the H-2 effect on leukemogenesis. (9) Although they had previously shown that intact FV particles do not possess FMR antigen on their surfaces, they have now found that when the virus particle is disrupted, there is a great deal of FMR activity detected. Most of this activity is still attached to fragments of the virus, because it is sedimentable by centrifugation at 100,000 G, but a portion of the activity is present in soluble form and can be recovered from Sephadex G 150 columns, mostly in large molecular from. Studies on the smaller molecular form of the antigen (25-35 thousand MW) obtained from infected spleen homogenates indicate that its isoelectric point is about 7.5-8.0; this is markedly different from that of the gs-1 antigen, which was about 5.5-6.0 in published studies.

Significance to Biomedical Research and the Program of the Institute:

One of the basic facts about tumor biology is that the genetic mechanisms of the host exert strong control over the expression of oncogenicity. This contract represents the only in-depth study being done on the genetics of susceptibility to viral oncogenesis. The knowledge derived from this study and the mouse strains developed will have broad applications both to the SALES program and the general effort in virus-cancer problems.

Proposed Course: The following experiments are planned for next year: (1) Comparison of further immunogenetic and viral parameters of infection by Friend virus in BALB/c and BALB.B mice. (2) Further studies of the polycythemia-anemia problem in BALB and C3H mice. (3) Absorption of F-S and F-B viruses by spleen cells of various mouse strains. (4) Attempts at final purification of the FMR antigen. (5) Mapping of the Fv-2 gene with respect to theta, mdh and Trf.

Date Contract Initiated: May 13, 1965

Current Contract Level: $123,990

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, MIAMI, FLORIDA (NIH-70-2211)

Title: Studies of the Rat Mammary Tumor Derived Virus

Contractor's Project Director: Dr. Victor V. Bergs

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: To determine whether purified C-type particle preparations (of BV) will induce leukemia or other malignancies when administered to conventional and germ-free rats under natural and immunologically altered conditions of the host; and (2) to determine whether the 9H virus (9HV), previously isolated from rat leukemia, will exert a helper or inhibitory effect upon co-infection with BV *in vivo* and *in vitro*.

Major Findings: The growth kinetics of RMTDV in cells of the established REL line of Sprague-Dawley rat embryo cells were determined. Following infection at a multiplicity of 1.0-0.01 TCID$_{50}$ per cell, the peak of the virus titer was reached by day 4 post-infection. High-titered (10^7 - 10^9 TCID$_{50}$/0.1 ml) RMTDV is now being routinely prepared from infected REL roller bottle cultures by sedimentation of the partially purified virus harvest at 78,000 x g. The virus is stored in 0.05M potassium citrate at -70 degrees C. for future use. Storage in 0.05 citrate caused an apparent aggregation of the virions of RMTDV, which segregated upon dilution with culture medium at 37 degrees C. Appreciable aggregation did not seem to occur in culture, and at the time the virus was harvested and sedimented, but only upon storage in 0.05 citrate at -70 degrees C; however, storage of RMTDV under these conditions is necessary to avoid marked loss of infectivity. (2) Infectious RMTDV banded at average buoyant densities of 1.12 and 1.25 gm/ml in a potassium tartrate gradient. Virus banding at a density of 1.25 gm/ml represented aggregated virions or virions attached to cellular components, RMTDV centrifuged in a preformed gradient of 50% and 10% sucrose by means of a Spinco SW 39 rotor at 38,000 rpm for 180 minutes, banded within a buoyant density range of 1.04 - 1.12 (average 1.08) gm/ml. If RMTDV was deliberately mixed with the 9H virus (9HV) and the mixture centrifuged under these conditions, RMTDV again banded within the above range; whereas 9HV banded at a density of 1.15 - 1.21 (average 1.18) gm/ml. These rebuoyant densities, and that the method was useful for separating these viruses. (3) High-titered (10^7 - 10^9 TCID$_{50}$) RMTDV was inoculated intraperitoneally into several groups of newborn Wistar/Furth and Sprague-Dawley rats 3-5 months ago, To date no grossly detectable biological effects have been observed in any of these animals; however, an early disease developed within 15-32 days in W/FU rats inoculated at birth with a mixture of RMTDV and 9HV. Histologically, it was characterized by focal coagulative necrosis and fatty change in the liver, accompanied by focal cell infiltrates which resembled leukemic cells of the lymphocytic type. Such a response did not occur in W/FU rats inoculated with either RMTDV or 9HV alone. In one experiment, animals inoculated with a potent preparation of 9HV combined with RMTDV, developed typical peliosis hepatis within 12-15 days, but none of the other changes. Control animals inoculated with 9HV alone also developed peliosis hepatis, and the ones inoculated with RMTDV alone showed no response. It appeared that in this case 9HV, because of its high potency, produced lethal peliosis hepatis before an expression of the changes caused by RMTDV occurred. Subsequent experiments showed that (a) 9HV antiserum blocked all the changes which may occur following a simultaneous inoculation of a mixture of RMTDV and 9HV as described above, and (b) RMTDV could be isolated from liver-spleen tissue extracts of animals that showed the focal necrosis, fatty change and cell infiltrate in the liver and had been previously inoculated with an RMTDV-9HV mixture. These extracts, however, did not cause any disease when inoculated into newborn rats, presumably due to the fact that infectious 9HV was absent or at low level as a result of high serum 9HV-antibody levels of the donor. (4) The WF line of Wistar/Furth rat embryo cells was established in their laboratory about 3 years ago. Recently cytopathological manifestations in this cell line were observed. Upon examination with the electron microscopie many typical C-type particles were seen budding from the cell membrane. Further investigations disclosed that lesions, consisting of transformed fusiform and rounded cells, appeared 4-7 days after seeding in culture vessels, provided the cultures were not refed. If the cells were refed or subcultured every 3-4 days no appreciable cytopathology could be detected. Similarly, if a culture showing some lesions was subcultured, the cells that grew out looked normal again, but such a culture again began to show altered cells after the 4th day if not refed earler. The WF cells were highly malignant, since 90-100% of the inoculated suckling W/FU rats developed rapidly growing tumors within 4-5 weeks and displayed infiltration of cells into vital organs. (5) A group-specific murine leukemia antisera, received from Huntingdon Research Center, Inc., failed to react significantly with RMTDV grown in REL cells in complement fixation tests. Since no positive antigen control was available, the results are not conclusive. These studies will be henceforth carried out in collaboration with Dr. C. Rickard.

Relationship to Total Program:

Although rats are highly susceptible to spontaneous cancers as well as to the induction of cancers by chemicals and irradiation, and C-type viruses similar to those which cause cancers in other species have been found in rats by electron microscopic studies, no such virus of rat origin has yet been proven to cause a cancer in this species. In addition to the important possibility of having a new laboratory model in another species for guiding approaches to the human virus-cancer problem, the proposed studies would also provide an important test of the Huebner hypothesis that C-type viruses are the determinants of most cancer in all species, including rats and humans for which oncogenic viruses have not yet been demonstrated.

Proposed Course: (1) Further in vivo and in vitro studies on co-infection with RMTDV and 9HV in regard to time and dose relationships. (2) Attempts to potentiate RMTDV by frequent serial transplants of leukemic cells and extraction of the resulting tumors for virus. (3) Attempts to potentiate the WF cell agent by serial in vivo passage of the cells, accompanied by inoculation of rats with cell free extracts of the tumors.

(4) Characterization of the agent present in the WF cell line and development of a suitable host system for its assay. (5) Further purification studies on RMTDV and subsequent characterization of its nucleic acid. (6) Studies on the possible infectivity of the nucleic acid of RMTDV.

Date Contract Initiated: June 22, 1970

Current Contract Level: $72,000

GERMFREE LIFE RESEARCH CENTER, FT. LAUDERDALE, FLORIDA (PH43-65-95)

Title: Germfree Research and Operation of a Collaborative Germfree Tumor Virus Laboratory

Contractor's Project Director: Dr. Joel Warren

Project Officer (NCI): Dr. W. Ray Bryan

Objectives: The activities under this contract are of three different types, each involving the special germfree and environmentally controlled SPF facilities and capabilities of the contractor: (1) Service type activities in general support of SVCP and intramural research requiring clean, defined, animals and viral reagents; (2) Participation in collaborative research primarily of other groups, but in which germfree and/or rigid SPF environmental control are required for definitive results; and (3) Research primarily by contractor scientists, but of mutual interest to the SVCP or collaborative with other SVCP groups where back-up in other special discipling are necessary. Specific objectives under (1) are: (a) Production of tumors in germfree avian and rodent animals by chemical carcinogens and propagation of such tumors by transplantation as a resource for studies of such tumors by other investigators. (b) Production of oncogenic viruses in germfree animals as a source of "reagent grade" virus for use in SVCP research, including that in this contractor's facility. (c) Supply of limited number of germfree animals to other investigators where feasible, as a spin-off from maintenance of the foundation colonies.

Major Findings:

Employing the helper-free, non-dependent RSV Beta-O strain of virus, they have completed a series of four replicate experiments designed to correlate the size of infecting dose with level of extractable virus in the ensuing tumors. There is a marked correlation between extractable virus and initiating dose. Regardless of the nature of the factors that might be essential for infectious virus replication within cells (including the cell-associated chf, and the virion-associated RNA-dependent DNA polymerase, the possibility exists that provirus can be integrated at multiple genetic loci, and that the amount of virus produced is quantitatively related to the number of such parasitic viral genomes incorporated per cell, as well as the number of cells embodying such productive intergrations, both of which might be expected to be related to input multiplicity of virus.

The amount of RSV in cell-free tumor extracts is highly dependent upon whether the host is germfree or not. Tobacco tar concentrate administered in a single dose will accelerate tumor formation with an associated increase in virus yield. These same experiments have uncovered the interesting observation that dimethylsulfoxide, administered into the breast muscle prior to inoculation of Rous virus, markedly increases the multiplicity of infectious virus in such tumors. Whether this effect is similar to that observed by others with DEAE remains to be established.

To determine whether a chemical carcinogen, methylcholanthrene, was active in a germfree, "C" particle-free host, a colony of Fischer rats was reared germfree and certified as "C" particle-free by the NIH. When 18 of such animals were inoculated with methylcholanthrene, all but one developed large tumors in approximately 100 days. One of these tumors, transferred through four generations in tissue culture, induced a sarcoma in conventional Fischer rats 73 days later. The cell-free extract of the culture is now on test in this host.

Work has continued on a comparison of properties of host-helper and virus-helper dependent properties of RSV strains. Using the low-dose technique of Bryan et al., they are investigating the A-variants of the Prague and Schmitt-Ruppin strains in germfree quail to determine whether the latter behave similarly to helper dependent RSV strain. They are also attempting to obtain the recently discovered RAV-0 agent by rapid passage of RSV tumors in quail. Further experiments substantiate the finding that RSV-0 multiplies to higher levels in muscle tissue of conventional animals than in germfree. Removal of birds from an isolator leads to rapid change in the host, resulting in increased virus proliferation in muscle. Similar experiments with non-helper-dependent RSV viruses are underway, as well as studies to determine whether the use of sterilized diets modifies the susceptibility of conventional animals.

A colony of germfree rodents has now reached an age at which spontaneous tumors are beginning to appear at an increasing rate. These, as well as MCA-induced neoplasms, are being studied in tissue culture in an attempt to isolate a transmissable agent.

Significance to Biomedical Research and the Program of the Institute:

The study of many of the most important problems in the virus causation of cancer in animals requires the use of certified animal hosts free of extraneous viruses and other pathogenic microorganisms, as well as skilled technical operations within environmentally controlled facilities, including completely germfree isolators for some purposes.

This contract is one of two established under the SVCP for supporting critical studies in such animals of interactions among viruses in dual infections (e.g., interference, enhancement, "helper" action), and co-carcinogenic activity between viruses and chemical compounds, both of which underlie the development of knowledge and technology for the detection and isolation of oncogenic viruses of man.

Future Course: To continue to develop, maintain, and distribute colonies of germfree and SPF mice, rats, and hamsters as required by SVCP projects. To continue to serve as the nucleus colony for other contracts and intramural laboratories. To continue the production of "reagent grade" lots of the Vogt strain of RSV in germfree quail for use in research in this and other SVCP contracts; appropriate bacteriological and histopathological monitoring will be continued. To continue the propagation of chemically induced tumors in germfree animals, investigate that, and supply either such tumors or the tumor-bearing animal to other participants in the SVCP. To continue to develop instrumentation for gnotobiology on a time-available basis. Particular attention is being given to aerosol and smoke chambers for germfree rodents. To test GF and SPF derived tissues *in vitro*. To maintain SPF animals on GF diets. To continue studies on determination of low dose response in DMSO-treated quail, and on the general usefulness of DMSO for viral isolation. To develop a pneumotropic strain of RSV-SR.

Date Contract Initiated: April 16, 1965

Current Contract Level: $197,266

LIFE SCIENCES, INC., ST. PETERSBURG, FLORIDA (PH43-69-63)

Title: Studies on Marek's Disease as a Model for Herpesvirus Associated Oncogenesis

Contractor's Project Director: Dr. Jack Frankel

Project Officer (NCI): Dr. W. Ray Bryan

Objectives: (1) to determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease, i.e. whether it is direct, or indirect (e.g. by interacting with some other agent, or by activating a covert viral oncogene--the Huebner hypothesis); (2) to establish Marek's disease as a model system for herpesvirus associated oncogenesis, using reagent grade SPF avian hosts (free of overt leukosis virus and other pathogens); and (3) to operate an avian virus testing laboratory for monitoring both hosts and viral materials used in experiments for freedom from extraneous viruses, including infectious leukosis virus.

Major Findings: (1) Techniques for extraction of MDHV in high titer from feather shaft base of MDHV infected chickens have been developed. (2) Detection of MDHV antigen in feather tips by agar gel double diffusion tests after two weeks coincided with the initial appearance of infectious MDHV. (3) MDHV neutralizing antibodies in response to infection were detected within six to seven weeks, and infectious MDHV could no longer be isolated at this time. (4) Electron microscopy during the period of MDHV isolation from feather shaft base consistently showed herpesvirus particles, inclusion bodies and small membranous particles to be present in the feather follicles. (5) Optimal conditions for assay of MDHV infectivity are: dilution in tryptose phosphate broth, absorption onto 48 hour old LSI-SPF chick embryo fibroblast (CEF) cultures (primary, secondary, tertiary), pretreatment with

50 mcg of diethylaminoethyl-dextran, and maintenance with tissue culture medium at an initial pH of 7.0. (6) CEF ($10^{3.7}$ FFu/ml) and chick kidney ($10^{3.5}$ FFu/ml) cultures and the CAM of LSI-SPF embryonated chicken eggs ($10^{3.2}$ PFu/ml) were found equally susceptible. (7) Titration of MDHV in LSI-SPF chicks showed that the pool contained $10^{3.2}$ LD_{50} per ml. (8) MDHV infectivity was retained following filtration through a 0.45u Millipore filter. (9) MDHV has been stored at -70 degrees for eleven weeks without infectivity loss. (10) Foci formation and pocks on the CAM were significantly reduced by serum derived from MD infected chickens. (11) Prior infection of CEF cultures with MD cells (tumor and CEF passage 5) significantly reduced plaque formation by influenza virus (strain WSN). (12) A monocontaminated research farm facility is in operation and provides an area for the housing of infected chickens and for studies involving natural exposure to MDHV. (13) The biocontainment facility (LSI, Building No. 3) containing eight Reyniers steel isolators, is available for studies with MDHV and other viruses. (14) The isolators prevented spread of a virulent virus (Newcastle disease, strain Roakin), from infected LSI-SPF chickens and quail to other birds in the outside environment and to birds housed in other isolators. (15) In the isolators, LSI-SPF and LSI germfree chicks responded to MDHV infection the same as LSI-SPF chicks housed at the monocontaminated research farm facility.

Significance to Biomedical Research and the Program of the Institute:

No satisfactory animal model is currently available for studying the role (i.e. whether direct of indirect) of herpes-type viruses in the induction of neoplasia. Since herpes-type viruses are associated in very high frequency with two types of human cancer, namely Burkitt's lymphoma and post-nasal carcinoma, an animal model system for developing approaches to and guiding studies on the human problem is urgently needed.

Since Marek's disease of chickens is also associated with a herpes virus (MDHV), and this virus can now be isolated and worked with systematically in the laboratory, it seems likely that this chicken disease can be developed into the desired laboratory model.

Proposed Course: (a) A variety of methods will be explored to increase the efficiency of extraction of MDHV from feather shaft bases and to stabilize MDHV infectivity during handling and storage. (b) The optimal conditions for detection of MDHV infectivity in tissue cultures and eggs will be determined and complement fixation and fluorescent antibody techniques will be developed. (c) Potent MDHV will be employed in interference and enhancement studies with a variety of viruses including viruses studied previously with MD cells. Studies with MDHV, herpes simplex and other herpesviruses will be emphasized. (d) The pathogenesis of Marek's disease in LSI-SPF chicks will be studied by sequential changes in histopathology, serology and virology. (e) Concentrated MDHV will be treated with formalin, ethylene oxide and beta propiolactone to determine the efficacy of inactivated vaccines to protect chickens against this herpesvirus.

Date Contract Initiated: November 1, 1968

Current Contract Level: $368,000

UNIVERSITY OF INDIANA, INDIANAPOLIS, INDIANA (NIH-69-2048)

Title: Characterization of the Twiehaus Agent of Avian Reticuloendotheliosis

Contractor's Project Director: Dr. Alvin S. Levine

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: (1) To increase Twiehaus virus production in tissue culture. (2) To develop an *in vitro* assay for Twiehaus virus, including immunofluorescence techniques. (3) To continue efforts to concentrate virus from infected bird tissues and possibly from tissue culture. (4) To continue studies on the immunological, physical, and chemical characteristics of Twiehaus virus. (5) To continue oncogenicity studies of Twiehaus virus in hamsters and to further define the potential of this virus to induce tumors in mammals.

Major Findings: Electron microscopic study of REV by thin-section and by the technique of negative staining (PTA) demonstrated the following ultrastructural characteristics: (1) A sequential disassembly of the structures of the virion was observed. (2) The virion appears to be triple-layered: a central shell; a dense homogeneous, intermediate layer; and an outer envelope bearing short, compact, fiber-like projections. (3) The overall diameter of the viral particle measures 750-900 Å in thin-section, and is estimated as 800 Å in the PTA preparations of virus receiving no prior treatment.

Reticuloendotheliosis virus was purified from liver and spleen homogenates and chick embryo fibroblast Rollacell cultures by a series of differential, velocity and sucrose density gradient centrifugation methods. This enveloped virus was found to have the following properties: (1) A diameter of approximately 100nm. (2) A buoyant density in sucrose of 1.16-1.18 g/cc. (3) Single-stranded RNA at an estimated concentration of 8 percent. (4) DNA polymerase activity.

Immunological studies on purified reticuloendotheliosis virus from infected chick embryo liver cell culture fluids gave the following results: (1) Sucrose gradient banded virus from chick embryo liver cell culture fluids did not cause death on injection into day old chicks. (2) The purified virus on inoculation into chick embryo fibroblast cultures gave positive immunofluorescence by the indirect method using fluorescein isothiocyanate labeled rabbit anti chicken gamma globulin. (3) Two non-chick antigen lines were obtained in immunodiffusion slides with chicken anti-reticuloendotheliosis virus sera. (4) Examination by electron microscopy of the denser of the two precipitin lines showed intact viral particles. (5) The denser of the two precipitin lines stained with Sudan Black B (lipid stain). (6) Enhanced immunocytolysis with rabbit anti-reticuloendotheliosis virus serum was observed in reticuloendotheliosis virus infected quail embryo fibroblasts. The antiserum was absorbed with normal chicken liver powder.

Tests to confirm induction of a tumor by reticuloendotheliosis virus in hamsters are in progress: (1) Eighty newborn hamsters were inoculated with a preparation of 20 percent homogenate of hamster growth having a chick ID_{50}/ml REV titer of <1 x 10^1. No tumors have been observed among 51 survivors. (2) Fifty newborn hamsters were inoculated with supernatant fluid from chick embryo liver tissue cultures made from nineteen day old embryos that were inoculated in vitro with reticuloendotheliosis virus. The infectivity titer of REV in the supernatant fluid was 2 x 10^3 chick ID_{50}/ml. No tumors have been observed among 37 survivors. (3) Forty-nine newborn hamsters were inoculated with supernatant fluid from chick embryo liver tissue cultures having an infectivity titer of REV of 9 x 10^3 chick ID_{50}/ml. No tumors have been observed among 34 survivors.

Collaborative studies are in progress with Life Sciences, Inc. under Contract No. PH69-63, to determine if any relationship exists between Marek's disease herpesvirus and RE virus. Preliminary data suggest that chicken REV immune sera from Indiana University (Levine) neutralized Marek's disease herpesvirus in vitro. The specificity of the test is not known. Gel diffusion tests using the above chicken REV immune sera did not give precipitin lines with MDHV antigen. Serum from an uninoculated contact control chicken caged with REV infected chickens neutralized MDHV in vitro, as measured by reduction of focus forming units and by production of a precipitin line against MDHV antigen in the gel diffusion test. The prevalence of MDHV in straight line Indiana Farm Bureau chickens is not known.

A survey of modes of horizontal transmission of reticuloendotheliosis virus, strain T, in chickens has been completed. The data show: (1) Titration of different organs (liver, spleen, lung, brain) from a specifically moribund bird shows the level of infectivity of virus is essentially the same throughout the chicken. (2) Individually, moribund birds vary widely in the titer of infectious virus recovered. A range from 1.85 to 5.5 ID_{50}/ml (log base 10) by titration of spleen-liver specimens was observed. (3) Feather dandruff was always found negative for infective virus. (4) Relative susceptibility of day old cockerels were tested. The most susceptible routes were found to be intramuscular subcutaneous, intraperitoneal, and intracardial. The least susceptible routes were found to be by the alimentary canal, intranasal, and intracranial. (5) Contact transmission studies show: (a) Simulated barnyard contact ratio of forty inoculated to forty uninoculated gave a 33 percent serologic conversion of the contact controls without evidence of reticuloendotheliosis in any bird but those inoculated. (b) Supported on wire above litter the contact ratio of forty inoculated to forty uninoculated gave a 13 percent serologic conversion of uninoculated. (c) Supported on wire above litter the contact ratio of one-hundred twenty inoculated to thirty uninoculated gave a 25 perent serologic conversion of the uninoculated. (d) Fifty uninoculated controls held in the same chicken room as the inoculated birds but not in contact (cages kept at a distance) over a period of forty-two days gave no evidence of disease or serologic conversion in the sera taken from thirty birds at random.

Significance to Biomedical Research and the Program of the Institute:

The avian reticuloendotheliosis virus is a leukemogenic virus prevalenting in many flocks of chickens, and is said to be transmitted both horizontally and vertically. The biologic characteristics and the understanding of the chemical, physical and immunological nature of the agent may be applicable to the possible role of viruses as etiologic agents in human neoplasia. Of equal importance is the determination of whether oncogenic viruses of animals have the capabilities to replicate and induce neoplastic diseases in man. The avian viruses must be evaluated for this potential.

Proposed Course: (1) Attenuation of REV(T) for vaccine strain(s). (2) Immunological comparison of REV(T) with other avian tumor viruses. Immunological study of antigen preparations from purified preparations of REV. (3) REV(T) with Marek's disease herpesvirus *in vitro* and *in vivo* to determine if interference or potentiation results. These studies are being carried out in collaboration with Life Sciences, Inc. (4) Characterization of REV polymerase(s) and the effect of specific REV(T) antibodies on polymerase activity.

Date Contract Inititated: May 8, 1969

Current Contract Level: $77,485

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, CORAL GABLES, FLORIDA (PH43-67-1187)

Title: Immunization Studies on Avian Leukosis and Related Problems

Contractor's Project Directors: Dr. M. Michael Sigel
Dr. Paul Meyers

Project Officer (NCI): Dr. John P. Glynn

Objectives: (1) Development of *in vivo* and *in vitro* assays for transplantation antigens. (2) Studies on protection against Rous-RAV-1 with small amounts of virus. (3) Attempts to purify tumor antigen derived from chicken tumors. (4) Studies on Rous tumor antigens obtained from hamster tumors.

Major Findings: (1) Successful immunization against tumor induction by RSV(RAV-1) can be achieved by immunization with either low doses of RSV (RAV-1) or with doses of RAV-1 in the range of 10^1 to 10^6 infectious units. Thus, the protection achieved by low dose RSV (RAV-1) immunization could probably be attributed to the RAV-1 component present in excess in stocks of RSV(RAV-1). (2) In every experiment in which SPAFAS chickens were immunized with low dose RSV(RAV-1) or RAV-1 there was a good correlation (about 90 percent) between resistance to tumor induction and neutralizing antibody. This correlation does not establish a causal relationship between antibody and protection, as there have been instances where protection was evident in the absence of antibody. (3) Protection can be achieved with a broad range of RAV-1 doses, but the critical factor in successful immunization appears to be elapsed time from start of immunization to challenge. Thus, only partial protection can be achieved when challenge is 20-30 days after the initiation of immunization, full protection requiring a lapse of at least 35 days. This requirement is probably compounded of time periods necessary for virus replication, production of protective transplantation antigens, and evocation of cellular immunity. Protection lasts at least 112 days, the latest time tested to date. (4) Immunization with RAV-1 offers significant protection (69 percent) against challenge with SR-RSV. Since these viruses are antigenically distinct, and there was no evidence that the immunizing or challenge viruses were other than pure subgroups A and B, respectively, (the RAV-1 immunized chickens had no neutralizing antibodies against SR-RSV, it has been tentatively concluded that cell-mediated immunity against a transplantation antigen induced by RAV-1 can effectively protect against tumor induction by heterologous virus. The putative transplantation antigen might therefore be considered group-specific for members of the avian leukosis/sarcoma complex. Further work will be pursued to define and expand this concept as it represents direct evidence of such a transplantation antigen in the natural host system. (5) Transient protection against SR-RSV hamster sarcoma cells has been evoked by immunization of hamsters with SR-RSV. Studies will be performed to define this system in the hope of achieving other than ephemeral protection. In addition, work on techniques for detecting cell-mediated immunity in this system (inhibition of macrophage migration) is progressing well and should yield valuable tools to approach the problem at a more precise level. Such work is now feasible by virtue of the development in their laboratory of a syngeneic Rous hamster tumor line characterized by high oncogenicity and the establishment of a tissue culture line of these cells.

Significance to Biomedical Research and the Program of the Institute:

The overall goal of the SVCP is the control of cancer. From the immunological viewpoint, it would be extremely important to clarify the issue as to the possible existence of a transplantation antigen and its role in the disease process. Basic studies are also needed on immunity in chickens in order to understand fully the immunologic implications in avian tumors, and possible application to mammals.

Proposed Course: The following are the major areas of investigation for next year: (1) Duration of immunity induced by immunization with live virus. (2) Expansion of the studies on specificity of the protective immunity using other subgroups of the leukosis system (heterologous combinations of immunizing and challenge viruses). (3) Humoral factors other than neutralizing antibody, such as the migration inhibition factor. (4) Cell-mediated immunity in hamsters and in chickens. In the former, a syngeneic SV_{40} model already developed will serve as a point of reference for the studies with the hamster Rous sarcoma system. In the latter, there is a need for obtaining basic information about the operation of cell-mediated immunity.

Date Contract Initiated: June 23, 1967

Current Contract Level: $47,645

UNIVERSITY OF NORTH DAKOTA, GRAND FORKS, NORTH DAKOTA (PH43-66-8)

Title: Quantitative studies on the transmission of feline oncogenic RNA viruses and selected herpesviruses by certain bloodsucking arthropods

Contractor's Project Director: Dr. Robert G. Fischer

Project Officer (NCI): Dr. George J. Burton

Objectives: To determine whether the following diseases induced by certain oncogenic viruses can be transmitted from infected animals to healthy susceptible animals by bloodsucking arthropods with special reference to virus quantitation: feline leukemia virus (FeLV), feline sarcoma virus (FSV), and murine cytomegalo virus (Henson), and Marek's disease virus. Specific objectives are: (1) To determine oncogenic virus levels in arthropods which have fed on experimentally infected donor animals of known titer (extrinsic incubation studies). (2) To determine whether the disease caused by each oncogenic virus can be transmitted biologically, i.e. by transfer of the virus via the salivary glands of the infected arthropod when taking a subsequent blood meal. (3) To determine the quantitative distribution of the virus in those arthropods demonstrating suitably high virus levels among various organs and tissues, including alimentary tract, hemolymph or blood, salivary glands, and in any other organ where virus may localize and multiply. (4) To determine whether the disease caused by each oncogenic virus can be transmitted mechanically through interrupted feeding, or by transfer of the virus on the legs and body of the arthropod (especially in Marek's disease).

Major Findings: It has been established that FLV could be transmitted by several insect species which had partially fed on an FLV-infected mouse and subsequently completed their blood meal on a "normal" suckling BALB/c mouse. The minimum number of bites necessary to initiate infection by Aedes triseriatus, Culex pipiens quinquefasciatus or Stomoxys calcitrans is one, while to date at least two bites by Anopheles quadrimaculatus have been necessary to initiate a leukemic response. The initial attempt at establishing the laboratory transmission rate was made and reported in Aedes triseriatus (5/114 or 4.34 percent), Anopheles quadrimaculatus (18/152 or 2.56 percent), Culex pipiens quinquefasciatus (6/114 or 5.26 percent), and Stomoxys calcitrans (18/152 or 11.84 percent).

In experiments involving Friend leukemia virus (FLV), several extrinsic incubation studies (i.e., determination of FLV fate) have been conducted with the black salt marsh mosquito Aedes taeniorhynchus, the malaria mosquito Anopheles quadrimaculatus, and the cat flea Ctenocephalides felis. In current studies, there was no indication of FLV activity beyond two days in any of the insects held at normal rearing temperatures (23 degrees C - 27 degrees C). It was seen earlier that cooler insectary temperatures (particularly 13 degrees C) slowed the rate of FLV decay but the overall response was not affected. Collaborative effort with Drs. W. Turner and G. Burton, N.I.H., has been initiated to determine whether the FLV complex ingested by Stomoxys calcitrans is altered with time in this fly, so that subsequent introduction of this insect-virus mixture into an animal system results in an occasional splenomegalic response without the concommitant formation of splenic foci. One group of 10,000 flies has been allowed to feed directly on intact viremic BALB/c mice; whereas the second group (6,000 flies) was fed from sterile sponges soaked with FLV virus-spleen-blood mixtures.

Extrinsic incubation studies of feline leukemia (lymphoma/lymphosarcoma) virus (FeLV) and feline fibrosarcoma virus (FSV) in the cat flea, Ctenocephalides felis have been performed in collaboration with Dr. C. Rickard, New York State Veterinary College at Cornell University and Dr. G. Burton, N. I. H. Groups of fleas have been fed through membranes in vitro on either FeLV-human blood or FSV-human blood mixtures on 9, 6, 3, 1, and 0 days before dissection.

In additional experiments, fleas fed directly on FeLV-viremic kittens in vivo were noted to ingest larger blood volumes (3X) than those which fed artificially through membranes. Groups fed on viremic kittens 9, 7, 3, 1, and 0 days earlier were used in additional studies.

Electron microscopic investigation on FeLV and FSV activity in fleas fed at various time periods (both in vitro and in vivo studies) is being done under subcontract to The Boyce Thompson Institute, Yonkers, New York, by Drs. H. Hirumi and K. Maramorosch. Approximately 200 sections, prepared from different parts of the flea digestive tract were examined. Blood cells, that were acquired by the feeding fleas together with the respective viruses, were completely broken up in the gut within a few hours. No virus particles were adhering to the stylets. Thus far, virus particles have not been detected in the materials infected with either feline leukemia or feline sarcoma virus because of the low titer in the blood. The examination of the remaining materials is in progress, dissected from fleas which had had in vitro and in vivo infected blood meals. The bone marrow, spleen and thymus have also been removed from the kittens for electron microscopic examination. If virus particles can be detected in these kitten tissues, the organs of fleas fed on them will be examined by electron microscopy. Initially, FeLV virus particles were seen in the bone marrow. An extensive study of the ultrastructure of normal cat fleas has been completed. This aspect will also be a valuable scientific contribution. Electron microscopy work was also started with the mosquitoes, Aedes aegypti and Anopheles stephensi, following post-feeding periods of 28-30 hours, 46 hours, 75-78 hours, and 19 days. Excised salivary glands, hindgut, midgut, esophagus, diverticuli, muscles, fatbody, feeding apparatus, ovaries, testes, and Malphighian tubules were fixed and embedded for electron microscopy examination.

Marek's disease transmission studies are being conducted with the collaboration of Drs. J. Frankel and V. Groupé of Life Sciences, Inc., St. Petersburg, Florida and Dr. G. Burton, N. I. H. An initial investigation has been carried out in order to ascertain which flying arthropod species were naturally present in or near infected chicken flocks. Studies involving laboratory reared insects (i.e., certain fleas, mites, and mosquitoes) have been initiated, making contact with the Marek's diseased chickens either by walking among the feathers, or by feeding on their blood. These studies are being carried out at the monocontaminated farm at Tarpon Springs, Florida. In order to determine whether certain herpes-type viruses can be transmitted by arthropods, mouse cytomegalovirus (Henson) was chosen for study during the 1971 contract year. Virus in infected salivary glands was supplied by Dr. D. Henson, N.I.H. and has undergone three passages at U.N.D. to produce material which induces consistently high viremias in mice to be used for future arthropod feeding. In February they will also collaborate with Drs. F. Deinhardt and L. Falk at Rush-Presbyterian-St. Luke's Medical Center, Chicago, on attempts to transmit herpes saimiri of marmosets by mosquitoes, flies and fleas.

Significance to Biomedical Research and the Program of the Institute: Arthropods have been investigated in relation to the transmission of human diseases for many years. Viruses, which are obligate parasites, may be transferred by arthropod vectors, and such transmission may occur biologically or mechanically. Arthropods such as moquitoes, biting flies, fleas, ticks and mites may suck blood from both man and from domestic animals (including birds) closely associated with man. It is, therefore, important to investigate whether leukemogenic viruses present in infected animals can be transferred to humans. If such vector or vectors are found, then measures can be taken to control the vector and to prevent contact between vector and human or animal hosts.

Proposed Course: If positive transmission results are obtained with herpes saimiri virus, then it is expected that priority will be given to investigations utilizing arthropods feeding on marmosets infected with this virus. As many species of common bloodsucking arthropods will be used as can be procured and reared in large numbers. Further investigations on Marek's disease virus, murine cytomegalovirus (Henson) and feline oncogenic viruses will depend on initial results.

Date Contract Initiated: October 27, 1965.

Current Contract Level: $106,786.

SOLID TUMOR-VIRUS PROGRAM SEGMENT

Dr. Robert J. Huebner, VCB, Etiology Area, Chairman
Dr. James T. Duff, VCB, Etiology Area, Vice Chairman

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (PH43-68-997)

Title: Role of Oncogenic Viruses in the Causation of Cancer in Man and his Domestic Animals

Contractor's Project Director: Dr. Edwin H. Lennette

Project Officer (NCI): Dr. James T. Duff

Objectives: To apply the newer knowledge of the nature of RNA viruses affecting murine species to the study of neoplasms of other species, namely domestic cats, dogs, and man.

Major Findings: Utilizing the indirect fluorescent antibody procedure, the infectious titer of feline leukemia viruses (FeLV) was determined and stocks of virus prepared for use in viral genome rescue studies of non-virus producing cell cultures derived from tumors of cats, dogs, and human. This technic has also been used to determine the neutralizing ability of serum against FeLV.

A survey was made of normal cat sera and the sera of cats with various types of malignancies for the presence of antibody against FeLV. Preliminary results indicate that approximately 5% of normal cats and 30% of cats with malignancies have antibodies to FeLV as determined by the indirect fluorescent antibody technic.

Studies with serum obtained from veterinarians utilizing the indirect fluorescent antibody procedure to detect antibodies to FeLV were completed on 698 sera obtained. Only one serum could be interpreted as positive using a human cell line infected with FeLV as the test antigen and the uninfected cell line as the normal control. A subsequent serum sample from this individual was negative in the test. It is thus evident that this "high risk" group do not produce antibodies against FeLV as determined by this technic. Samples of these sera have been submitted to NCI laboratories for complement fixation studies.

In order to facilitate detection and assay of transforming feline sarcoma viruses, an attempt was made to increase the susceptibility and uniformity of focus-formation in beagle (dog) cells by rapid subconfluent passage of a line of beagle embryo cells. The resulting cell line (rapid passage) consistently produces countable foci at endpoint dilutions with both the Gardner-Arnstein and Snyder-Theilen strains of feline sarcoma viruses.

Proposed Course: (1) To continue utilizing these technics to determine if an immunological relationship exists in tumors of different animals and different species. (2) To continue to establish cell cultures from tumors of different animals and attempt to rescue a defective viral genome from such cultures.

Significance to Biomedical Research and the Program of the Institute: These studies are an integral part of a comprehensive field program on the etiology of cancer being carried out as a collaborative effort between the Viral Carcinogenesis Branch, NCI, the California State Department of Public Health (NIH 69-87), the University of California (Naval Biomedical Research Laboratories, PH43-63-13), the University of Southern California (PH43-68-1030), the Los Angeles Children's Hospital and the Los Angeles County Health Department. To a large extent the success of these programs depends on the acquisition of fresh human and animal (pet) cancer materials and tissue cultures derived from donors of these species having varying genetic backgrounds.

Date Contract Initiated: June 24, 1968

Current Contract Level: $220,000

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (NIH 69-87)

Title: Human-Feline Cancer Household Study

Contractor's Project Director: Dr. Robert Schneider

Project Officer (NCI): Dr. James T. Duff

Objectives: The purpose of this study is to determine if an association exists between human and feline cancer. The primary study is a retrospective one, utilizing personal interviews of case and control households initially identified in the Animal Neoplasm Registry (ANR). Case households are those in which a malignant cancer has been diagnosed in a cat. Control households are of two types: the first contains cats that have not had cancer and the second comprises non-pet-owning ones for at least 5 years prior to interview. Other related epidemiological studies also are being carried out from data available in the ANR, the Alameda County Tumor Registry (Human) and the household interviews.

Major Findings: Preliminary analyses have been completed on the 675 case and control households as to whether there was increased human, canine, or second cat cancers in households containing index cats with cancer. No evidence for transmission of feline oncogenic agents to man and dogs or between cats was found. Canine tumor numbers were 30 and 33 in case and control groups, respectively. There was no pattern of dog tumors with the feline leukemia-sarcoma complex; many different types of canine tumors occurred. Human varified tumor numbers of 75 and 57 in case and control households, respectively, did not differ significantly after adjustments were made for human population age difference in the two groups. As with the dogs, the overall human tumor numbers in the case group were proportionately distributed among the various cat cancer types. There were nine human leukemia-lymphomas of various types and one sarcoma diagnosed in association with cat cancers; however, there was no pattern with these occurrences. In 27 of the 675 case households, two or more cats had confirmed tumors during a 6 year period. Malignant lymphoma was the cancer found most often, either in both cats in the same household or in association with other tumors. The high prevalence of malignant lymphoma was found due to it being the principle cancer of cats and its occurrence in high frequency in young cats. Testing the eight observed two-cat combinations of the leukemia-lymphoma group indicated that the number was not significantly different from the 6.6 expected. Other tests carried out on this data reinforced these negative results.

Proposed Course: The epidemiological survey will continue in order to follow the occurrence of cancer in households that have been identified and to follow any leads that come from the initial study.

Significance to Biomedical Research and the Program of the Institute: This is the only population based animal tumor registry in the world, at present. It has been functioning since July, 1963. All cases are histopathologically confirmed. A case record is present for each animal, which, in addition to animal and tumor data, contains the owner's name and address. Thus, human contacts for specific animal cancers can be identified and sera can be obtained from such persons for serological testing. In addition the ANR obtains fresh animal tissues, animal fetuses, and live cancerous animals for the other contracts within the NCI Special Virus Cancer Program. This epidemiological study is extremely relevant to determining the relationship of the cat leukemia virus to human cancer and also for determining the role of horizontal spread of virus from cat to cat and from cat to dog.

Date Contract Initiated: June 19, 1969

Current Contract Level: $33,000

FLOW LABORATORIES, INC. (NIH 71-2097)

Title: Virus Laboratory for Cancer Research

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct immunological and serological studies of oncogenic B- and C-type RNA viruses and the herpes-like viruses associated with neoplasia. The current emphasis is on the isolation and characterization of the structural subunits of these viruses, to determine not only the interrelationships among viruses from several animal species, but also the possibility of finding similar proteins in human material.

Major Findings: The group specific proteins of cat, mouse, and hamster C-type viruses was isolated from disrupted virus by isoelectric focusing and specific antibodies were prepared in guinea pigs. Like the mouse, hamster, and rat, the cat gs protein contains both species specific and interspecies reactive antigenic determinants (interspec). The rat C-type virus gs protein has also been isolated by electrofocusing based on interspec reactions. Guinea pigs have been immunized with this purified protein fraction. Specific gs antisera, conjugated with fluorescein isothiocyanate, have been used successfully to stain infected cultures. The pattern of specificity seen in gel diffusion assays was upheld in fluorescent tests. The gs protein was clearly separated from the virion DNA polymerase activity in isoelectric focus separations, and enzyme active fractions have been used to immunize rats. Specific antibody capable of neutralizing the polymerases of mammalian C-type viruses was demonstrated. Polymerase activity was also demonstrated in simian and feline syncytial viruses. These viruses were negative in tests for interspec. A number of leukemic bone marrow aspirates (17/25) were able to polymerize dGTP using poly I:C as substrate. Activity was greatest in tween-ether treated cell pellets. Among cell lines tested, several human tumor derived lines appeared positive whereas normal cells, Burkitt lymphoma cells, and non-producer MSV hamster tumor cells were negative. These results obviously are restricted to the assay conditions used and could reflect quantitative differences in a normal cell enzyme (e.g., DNA polymerase).

Permanent hybrid cell lines were produced between a mouse cell line, with and without Rauscher leukemia virus, and MSV non-producer hamster cells. Those previously infected with Rauscher virus yielded sarcoma virus shedding cultures, whereas no virus was detected in cells not previously infected. Thus, presence of the MSV genome in normal mouse cell is not sufficient to induce virus synthesis.

The 7S fraction of human Herpes-like virus (HLV) positive sera stains HLV capsids by immunoferritin, whereas the 19S fraction of infectious mononucleosis sera stains HLV capsids. Based on previous findings with Herpesviruses, a close relationship between infectious mononucleosis and HLV is suggested. Comparison of immunofluorescent (FA) and immunoferritin staining characteristics of HLV infected cells by 7S and 19S fractions of various sera shows considerable deviation from the generalization that FA staining of fixed cells detects capsid antigens, whereas live cell staining reflects enveloped antigen.

Contracts NIH 70-2015, PH43-67-1396, and NIH 69-97 were combined into a single contract on February 1, 1971. Summary reports for contracts 70-2015 and 67-1396 are included under the Program Management (70-2015) and Sales (67-1396) Segments.

Proposed Course: (1) Structural analysis of the gs proteins from viruses of mouse, cat, hamster, and rat origin.
(2) Search for similar proteins in other species.
(3) Purification and characterization of virion polymerases.
(4) Characterization of polymerase activity in tumor cells.
(5) Extension of hybrid cell techniques to human cell systems.
(6) Continued definition of Herpesviruses (especially HLV) reagents.

Significance to Biomedical Research and the Program of the Institute: This project bears importantly on a main problem associated with determining whether viruses recovered from man, and which may be oncogenic for laboratory animals, are also oncogenic in man. A main emphasis of the Viral Oncology Program is to detect, isolate and identify viruses from human cancer patients. Since man will not be used as an experimental recipient, it is necessary to gain proof of oncogenicity by other means including seroepidemiological surveys for virus, virus antigen, and/or specific antibody. The detection of a common antigen among known mammalian C-type viruses would provide a powerful incentive to look for similar antigens in human material.

Date Contract Initiated: February 1, 1971

Current Contract Level: $2,400,000

MINNESOTA, UNIVERSITY OF (NIH 69-2061)

Title: Tumor-Specific Transplantation Antigens in Solid Tumors

Contractor's Project Director: Dr. Charles F. McKhann

Co-Project Investigators: Dr. K. E. Hellström (Univ. of Wash.)
Dr. J. David (Robert B. Brigham Hospital)

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To search for and characterize serum-mediated and cell-mediated immune responses to tumor-specific antigens in human tumors.

Major Findings: University of Minnesota (McKHANN): (1) Developed growth inhibition *in vitro* assay for cell-mediated immunity, which appears to work well in the case of both animals and humans. The presence of blocking antibody was demonstrated with this technic in a patient with Rhabdomyosarcoma; (2) An assay for a cytotoxic antibody was developed based on inhibition of thymidine incorporation; (3) *In vitro* immunization of normal mouse spleen cells against two syngeneic methylcholanthrene induced sarcomas was carried out successfully. The immunized spleen cells were cytodestructive when incubated with labeled target tumor cells.

University of Washington (HELLSTROM): (1) Two extremely significant facts were established in over 200 patients with a variety of histologic types of cancer: (a) Human tumors of the same histologic type have common antigens. Lymphocytes from a given cancer patient will inhibit his own tumor cells in tissue culture and also tumor cells of other patients of the same histologic type, but not of different histologic types; (b) human tumors of the same histologic type have common blocking antibodies. The serum from a given patient will block the tumor-destructive effect of his own lymphocytes for his own tumor and also for tumors of the same histologic type from other patients. (2) Six publications occurred in the contract year on blocking and nonblocking serum effects of cellular immunity to both animal and human tumors. Cell mediated immunity against antigens common to human colon carcinoma and fetal gut epithelium was demonstrated by the colony inhibition assay.

Robert B. Brigham Hospital (DAVID): Continuing studies on the guinea pig hepatoma system have shown that the interaction of sensitized lymphocytes in tumor cells result in the release of MIF and that irradiation or trypsinization of tumor cells does not destroy this effect. Separation of guinea pig

MIF from lymphotoxin was achieved.

Significance to Biomedical Research and the Program of the Institute: This joint effort from three different laboratories in separate institutions represents a coordinated effort to screen large numbers of human tumors for tumor-specific antigen and to characterize the cellular immune response in humans. The success of this effort opens the way for the development of in vitro assays to study the cancer patient's immune responses to his own tumor.

Proposed Course: To continue to search for and characterize serum-mediated and cell-mediated immune responses to tumor-specific antigens in human tumors.

Date Contract Initiated: April 14, 1969

Current Contract Level: $180,500

SAINT LOUIS UNIVERSITY (PH43-67-692)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by RNA and DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct studies of the basic mechanisms of viral oncogenesis with both DNA and RNA tumor viruses and of the relationship between these viruses and human cancer.

Major Findings: The murine sarcoma virus (MSV), feline sarcoma virus (FSV), and avian myeloblastosis (AMV) virus RNA dependent and DNA dependent DNA polymerases and their products were characterized. The whole viral genome appears to be copied as small DNA molecules, 5-7S, 150,000 daltons in molecular weight.

The two DNA polymerase activities of RNA tumor viruses can be inhibited independently by the use of modified ansa ring structures (e.g., rifampicin derivatives). The formation of an RNA-DNA hybrid by MSV, FLV, and AMV utilizing the endogenous viral RNA template is inhibited by rifampicin itself; the formation of duplex DNA's is inhibited by actinomycin D. These and related compounds potentially provide a rational approach to the chemotherapy of viral diseases and possibly cancer.

In contrast to previous beliefs, cells transformed by RNA tumor viruses transcribe large amounts of viral RNA--as much as 5% of the nuclear RNA and 0.5-1% of the cytoplasmic RNA is viral specific. Molecular hybridization coupled with hydroxyapatite chromatography has shown that even cryptically transformed cells, which possess no detectable infectious virus nor virion proteins, transcribe viral RNA sequences. This ultrasensitive technic provides a method for detecting small amounts of viral genetic information in transformed cells and human cancer cells.

Viral gene expression is controlled at two levels: (1) transcriptional and (2) post-transcriptional. Early after infection with Ad 2, 3 viral RNA's 17, 23, and 27S are transcribed in the absence of protein synthesis by the cell's DNA dependent RNA polymerase. Late after infection, four large Ad 2 RNA nuclear species of 36 to 43S are rapidly synthesized and appear to be precursors of six to eight stable viral RNA species in the polyribosomes. The Ad 7 genome is transcribed sequentially during productive infection. The fraction of the viral genome transcribed early, late, and in transformed cells was established and individual viral RNA molecules were resolved. However, individual viral RNA molecules were not resolved from Ad 2 transformed rat cells. Instead, large heterogeneous viral RNA-cell RNA hybrid molecules appear to be transcribed from integrated viral DNA sequences in the cell chromosome. Ad 2, 7, and 12 transformed cells were shown to have 14-97 viral DNA copies associated with cellular chromosomes.

Over 200 human cancer RNA specimens were analyzed and found to contain less than 1,000 viral mRNA molecules specific for Ad 2, 7, and 12.

The summer adenocarcinoma of the frog, which contains no detectable herpes-like particles transcribes herpesvirus specific RNA, thus providing strong evidence for the viral etiology of this tumor.

Proposed Course: A comprehensive program studying RNA tumor viruses will focus on four major areas: (1) properties and biological function of the RNA dependent DNA polymerase of the leukemia-sarcoma virus. (2) The mechanism of RNA tumor virus replication and cell transformation. (3) The properties and function of gs antigen in virus infected, transformed, and embryo cells, and (4) the application of basic information on RNA tumor virus replication and cell transformation to the analysis of human cancers. Specific inhibitors of the RNA tumor virus RNA dependent DNA polymerase will be studied and evaluated as to their usefulness for a rational chemotherapy of virus diseases and cancer. In addition temperature sensitive mutants of MSV and adenoviruses will be used to analyze viral gene functions involved in cell transformation.

Significance to Biomedical Research and the Program of the Institute: Within the Special Virus Cancer Program sequential scientific activities, which must be conducted prior to the development of a means for the prevention of virus-induced neoplasia in man, include (a) detection of the virus or virus product in human materials, (b) identification of the virus as a known or new agent, (c) selected biochemical characterization of the agent, and (d) verification of oncogenicity for man. The basic research on the molecular biology of normal and virus infected cells may provide the basis for understanding the mechanism of animal virus infection and carcinogenesis. These studies provide the basis for developing a rational chemotherapy for viral diseases and cancer.

Date Contract Initiated: March 20, 1967

Current Contract Level: $750,000

SALK INSTITUTE (PH43-67-1147)

Title: Characterization of Temperature-Sensitive Mutants of Polyoma Virus, and Interaction Between Polyoma Virus and C-type RNA Viruses

Contractor's Project Director: Dr. Walter Eckhart

Project Officer (NCI): Dr. George J. Todaro

Objectives: To conduct studies on the characterization of temperature-sensitive mutants of polyoma virus that are defective in functions required for cell transformation. Interactions between polyoma virus and C-type RNA viruses are being studied to determine whether any polyoma virus function can activate the expression of a C-type RNA virus genome. Initiate studies on the isolation of cells transformed by murine sarcoma virus that are temperature-sensitive in their transformed phenotype.

Major Findings: Studies have continued on temperature-sensitive mutants of polyoma virus to identify the viral functions required for transformation. Studies with these mutants have identified two viral gene functions required for transformation. One is required transiently to initiate or stabilize the transformation, the other is required continuously to maintain certain aspects of the transformed phenotype, particularly, cell surface alterations that affect cell growth control.

The majority of these studies are concerned with the ts3 mutant, which is defective in a gene function required for induction of cellular DNA synthesis in resting Balb/3T3 cells, and for initiating and maintaining surface alterations detected by agglutination of infected or transformed cells by wheat germ agglutinin or concanavalin A. It was found that the ts3 mutant neither relieves the cells of a serum factor requirement, nor alters the surface in such a way as to break contact between cells to allow expression of virus induced DNA synthesis. It is possible that the ts3 gene function may act directly on the DNA synthesizing machinery of the cell. Several kinds of ts3 transformed cells have been selected to test specific ideas about the chemistry of surface changes and stabilization of viral genes in transformed cells. Attempts are being made to clarify the genetic relation between mutants defective in functions required for transformation and other mutants of polyoma, such as host range mutants.

Proposed Course: (1) Determine whether more than two viral genes are involved in transformation. (2) Identify the surface alterations of transformed cells that are important for cell growth control. (3) Characterize the "activation" function of polyoma that is responsible for induction of cellular DNA synthesis after infection and that appears to be involved somehow in the cell surface changes and cell growth alterations that take place after infection. (4) Determine whether any of the ts mutants of polyoma virus affects the expression of antigens associated with the C-type RNA viruses and attempt to relate the functions of the two different kinds of viruses.

Significance to Biomedical Research and the Program of the Institute: Polyoma virus can infect certain cells and cause them to be stably transformed so that their phenotype resembles, in many respects, that of tumor cells. It should be possible to define the genome of these viruses, including those genes responsible for the establishment and maintenance of transformation, by isolating temperature-sensitive mutants. An understanding of the molecular mechanisms of virus transformation should also throw light on the essential differences between normal and cancerous cells.

Date Contract Initiated: June 5, 1967

Current Contract Level: $45,000

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF and CHILDREN'S HOSPITAL OF LOS ANGELES (PH43-68-1030)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
and Dr. Robert M. McAllister
(Children's Hospital)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: A multifaceted, highly interrelated effort to determine the actuating and contributing roles of viruses, physical and chemical carcinogens, as well as other factors associated with cancer development, both individually and in concert, to the etiology of human and animal cancer in a natural urban ecology.

Area I - Project A (USC): The collection, distribution, growth, and study of specified human and animal cancer and control materials, as well as tissues derived from genetically defective individuals and spontaneous and therapeutic abortions.

Area I - Project B (Children's Hospital): In vitro studies involving materials derived primarily from cancer patients, as well as those whose genetic deficiencies are associated with a high incidence of cancer. Primary emphasis is focused on transformation of human cultures in an effort to rescue the human C-type virus genome.

Area II - Epidemiology: Studies of the incidence and prevalence of contemporary cancers in relation to (a) exposure to environmental pollutants; (b) possible genetic or ethnic influences; (c) dietary and other cultural and individual patterns, which are suspected to have possible cancer-inducing properties (coffee and tea, smoking, incense, hormone therapy (the pill), immunosuppressants, etc.); (d) development and organization of hospital based registries; and (e) computerization of data for USC and NCI use.

Area III - Environmental and Ecological Studies: Studies to measure relative exposures to environmental pollutants on a residential and occupational basis, and as determined by leads developed in Areas I and II of the program. Materials collected are characterized and disseminated for study in animal and tissue culture systems at the USC and other collaborating laboratories (VCB, NIH 70-2068).

Area IV - Co-carcinogenesis Studies: Studies to determine effects of naturally occurring environmental carcinogens in various animal species, with particular emphasis on free-living animals (wild mice, cats, etc.), which share man's ecology.

Major Findings: Area I- Projects A and B. Resources. Two hundred twenty-eight human tumors and 68 human fetuses (from spontaneous and therapeutic abortions) were obtained and processed in the period July 1, 1970, to March 15, 1971. Tumors included 24 sarcomas, 5 lymphomas, 155 carcinomas and 44 benign conditions. Five non-tumor specimens of interest (i.e., rejected transplanted kidney) were processed for study as well. (Thirteen specimens, not included in the totals, comprised muscle and nerve biopsies performed for diagnostic purposes.) Sera was obtained on 91 patients (38%) and normal tissue from 79 (33%).

In other categories 83 field cats and four dogs, carrying a variety of tumors, were referred by agreement with local veterinarians.

In addition to on-site serological, immunological, biochemical, and electron microscopy studies, human and feline tissue extracts were sent to the Viral Carcinogenesis Branch, NCI, for serology; and selected tissues were sent for culture and study to the Naval Biomedical Research Laboratory (PH43-63-13), the California State Department of Public Health (PH43-68-997), Flow Laboratories (NIH 71-2097), and other contract and collaborating laboratories, as appropriate.

Serology and Immunology. Positive complement fixation reactions in a large number of human tumor and fetal tissue extracts with several MSV rat sera pools suggest the presence in these tissues of an interspecies cross-reacting gs antigenic component. Comparable CF reactions with one human cancer sera suggest a possible break in immunologic tolerance in this patient to this particular antigen.

Several human tumor culture supernatants showed H^3 uridine uptake at 1.16 density in a sucrose gradient. Evidence of virus particles or virus activity in the primary tumors or gradient bands has, however, not yet been demonstrated. Study of C-type virions shed from human RD sarcoma cells following *in vitro* infection with FeLV and, on occasion, following transplantation into fetal cats, continues in an effort to demonstrate possible rescue or activation of a human sarcoma genome, either by producing sarcoma in cat fetuses, producing transformed foci in cat, dog, and human cell cultures, or showing different electrophoretic mobilities of the denatured virion RNA compared to the input

FeLV RNA. RNA dependent DNA polymerase activity was demonstrated in the virions shed from the RD cells and the DNA product of the viral genome was hybridized with specific RNA from the FeLV infected human RD sarcoma cells.

Broadly reactive MSV rat sera pool 21 and FSV dog antisera generally react in CF with tissues from those feline conditions most commonly associated with C-type viruses, namely spontaneous lymphoma, infectious peritonitis and anemia, and experimental FSV sarcoma. We have been unsuccessful, so far, in isolating additional feline RNA tumor viruses by inoculation of cat fetuses with several naturally occurring feline sarcomas and carcinomas, some containing demonstrable C-type particles. The DNA polymerase activities of FeLV and FSV using natural and synthetic templates were studied. The DNA directed enzyme activity of FSV with a synthetic duplex required simultaneous copying of both template strands of a homopolymeric duplex. Nearest neighbor analyses of DNA products of representative feline, avian and murine RNA tumor viruses suggest interspecies variation in the nucleotide sequence of the RNA genome copied _in vitro_.

Feline Studies: Studies of spontaneously occurring cancer in the cat have revealed an unusual predilection for oral cancer, which, in view of feline grooming habits, raises the question of possible carcinogen induction (e.g., smog particulates collected on the coat and transported via licking to the mouth and tongue).

Serological tests indicate there is good correlation between tissues containing C-type particles and positive reactions in the complement fixation test.

Studies to determine possible horizontal transmission of feline cancer cells or leukemia viruses by the common cat flea have proved negative, although it has been ascertained that cells could technically be transferred from one cat host to another. In addition examination of two of seven modified live vaccine products grown in feline embryo tissue cultures for possible contamination with the feline leukemia virus proved negative for infectious virus and in the electron microscope.

A cat breeding program at a nearby reformatory has been very successful and promises to provide the bulk of cats needed by the program.

Area II - Epidemiology and Cancer Surveillance Program: The cancer surveillance program is being developed to utilize a rapid reporting system from hospital pathology and hematology laboratories. Pilot efforts are now under way in three large hospital centers--University of Southern California Medical Center, UCLA Medical Center, and the Queen of Angels Hospital. Cases being referred will be utilized initially to study the relationship of several factors including (a) maternal and paternal age of cancer patient, (b) birth order, (c) family history of cancer, allergic conditions, diabetes, and congenital abnormalities, and (d) ethnic background to the risk of cancer. A questionnaire is being devised and will be field-tested shortly.

Area III - Environmental Studies: Four sampling trailers, located at defined high and low smog areas, are now in full operation and a comprehensive fractionation has been started on an annual composite of organic extracts of airborne particulate matter. When separations are complete, fractions will be distributed for biological testing. Crude fractions have already been tested in vitro under another contract (NIH 70-2068) and were found to produce cell transformations.

Area IV - Co-carcinogenesis (in vivo): Using broadly reactive MSV rat sera pool 21, gs antigenic expression has been commonly detected in different tissues from freshly trapped, untreated wild house mice never housed in the laboratory. C-type and intracisternal A-type particles are also seen on occasion in many of these tissues. This indicates that the C- and A-type viral genomes must, indeed, be ubiquitous in this natural feral species. However, infectious C-type virus has not yet been isolated from any source despite all the more recent virus isolation procedures. B-type virus particles were seen in breast tissue from two normal lactating wild mice never housed in the laboratory. A polyoma virus infected ecology of wild mice has been defined and will be studied for RNA tumor virus expression with aging in comparison with polyoma free mice. Attempts to activate infectious virus by UV irradiation from a Ki-MSV transformed non-productive rat cell line and monocellular clones and from spontaneous transformed foci of rat cells were unsuccessful.

Proposed Course: Area I - Projects A and B: In addition to ongoing studies, Area I, contract 68-1030 (projects A and B) has been expanded to include two biochemical units, which will focus on the following problems: (1) the role of the C-type RNA viral genome and the RNA-dependent DNA polymerase in normal embryonic cell growth and differentiation, (2) to determine the presence or absence of double-stranded viral RNA and the RNA-dependent DNA polymerase or their products in human and animal cancer cell lines, (3) attempts to isolate and characterize possible inhibitors or repressors of RNA tumor virus expressions, (4) attempts to characterize virus derepressing mechanisms of chemical carcinogens, and (5) determine whether components of a human sarcoma virus genome are incorporated into human sarcoma cell lines infected with animal C-type viruses and utilize animal model system cell hybridization technics in efforts to detect and rescue components of the human C-type RNA genome and its associated polymerase.

The serological unit is being expanded to accommodate the increased specimen load. An immunology unit has been established; its initial focus will be directed to isolating human C-type RNA particles or viral genomes utilizing immunological procedures, which proved successful in unmasking covert animal cancer viruses. Methods to be employed include (1) screening of a variety of tissues from genetically susceptible individuals with leukemia, stimulating the leukemic lymphocytes with phytohemagglutinin (PHA) in efforts to detect the human C-type genome, (2) performing parallel studies in human and animal tissues from a variety of age groups, (3) combining hydrocarbon carcinogens with PHA in efforts to "activate" viruses and transform lymphocytes, (4) establishing long-term lymphocyte cultures from selected patients, and (5) culturing animal tumor viruses in long-term and PHA-treated lymphocyte cultures.

Area II - Epidemiology: The epidemiology program, designed to gain information on the contemporary occurrence of cancer within several defined subenvironments of Los Angeles County is being expanded to provide back-up service and training for a proposed county-wide cancer surveillance registry. When fully implemented, it is estimated that Project Directors will have access to most of the major hospitals in the Los Angeles area, covering 70-80% of all cancer patients including their physicians and families and their medical, residential, and occupational histories.

Area III - Environmental Studies: At the present time facilities for sampling four reasonably distinct areas of Los Angeles County in terms of air pollution are in full operation. Smog specimens are collected, concentrated, and disseminated for *in vivo* and *in vitro* studies at USC and in related contract (NIH 70-2068) and NCI laboratories. Characterization of components has been expanded from benzopyrene and various gases to a number of additional hydrocarbon and tar constituents.

Area IV - Co-carcinogenesis: Proposed studies are in line with original objectives to explore the interaction of chemical carcinogens with the C-type viral genome, particularly in the cat and feral mouse. Atmospheric residues, demonstrated by Freeman, et. al., to be carcinogenic *in vitro* (collected and distributed by Area III air sampling program) will be utilized to determine their effects *in vivo* and *in vitro* under a variety of host, host cell, and environmentally defined circumstances.

Significance to Biomedical Research and the Program of the Institute: This program concerns itself directly with the search for causes of human, pet, and other animal cancers in a natural diversified ecology, utilizing (1) the technology developed in experimental animal cancer systems, (2) the information developed on genetic influences, cell susceptibilities, etc., (3) relevant chemical and physical carcinogenesis studies, and (4) epidemiological approaches designed to exploit Los Angeles resources, and to obtain a profile of cancer incidence in humans and animals, which share their ecology, in relation to exposure, to known and suspected environment carcinogens.

In addition the USC program continues as a prime resource for supplying human and animal materials to the SVCP in-house and contract programs, in general, but particularly to VCB contract groups (Microbiological Associates-- NIH 70-2068, Flow Laboratories--NIH 71-2097, St. Louis University--PH43-67-692, Naval Biomedical Research Laboratories--PH43-63-13, and the California State Department of Public Health--PH43-68-997).

Date Contract Initiated: June 26, 1968

Current Contract Level: $1,600,000

STANFORD UNIVERSITY (NIH 69-2053)

Title: Procurement, Processing, Storage, Distribution and Study of Human Tumor Cell Cultures and Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Director: Dr. Leonard Hayflick

Project Officer (NCI): Dr. James T. Duff

Objectives: Part A is for the procurement, processing, distribution and study of human tumor cell cultures. Serum samples and skin punch biopsy material will be collected from the patient and will be available to recipients of the tumor material, if necessary. In addition to the characterization and distribution of these materials, research studies will be directed toward the detection of a viral genome in these cells. The human tumor material is obtained primarily from hospitals in the San Francisco Bay Area and other collaborating contractors in the Special Virus Cancer Program.

Part B serves as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera cell cultures and clinical materials submitted by other SVCP contractors. Upon request, virological identification of isolates is made as to species and mycoplasma antigens are distributed to those investigators requiring these materials.

Major Findings: Various human tumors are being cultivated, photographed *in vitro*, and grown in sufficient quantity to deposit in ampoules in liquid nitrogen for use by this and other collaborating laboratories.

Several chemical carcinogens are under study for their ability to transform normal human cells *in vitro*; toxicity studies are now completed.

Differences in membrane properties of human, normal, and tumor cells are being studied; enzyme studies are now in progress.

Cells from several human tumors have been grown in mice treated with antilymphocytic sera. Normal human cells do not grow under these conditions. ALS treated mice can apparently discriminate between malignant and normal cells. Inhibition of HeLA cell growth *in vitro* by WI-38 has also been observed.

Five hundred twenty-five samples, submitted by various NCI collaborating scientists, were tested for mycoplasmas between October 1, 1970, and February 1, 1971 and found to be positive.

Collaborative studies were undertaken with Drs. Todaro and Aaronson on isotope labelling and density gradient separation of mycoplasma contaminants in cell cultures. All species, except *M.salivarium*, can be detected by this method. Quantitative aspects are now under study. Studies were completed on the sterol requirements of the T-mycoplasmas. Proof of a sterol requirement for the T-mycoplasmas and several other properties of these unusual micro-organisms suggests they be regarded as a new genus in the Class Mollicutes.

Proposed Course: (1) Continuation and expansion of collection, cultivation and characterization of human tumor cells. (2) Baking of human tumor cells in LN_2. (3) Attempts to transform normal human cells with RNA tumor viruses. (4) Continuation of studies on (a) effects of various chemical carcinogens on normal human cells *in vitro*, (b) quantitation of isotope labelling technic for mycoplasma detection and identification, and (c) discrimination by animals treated with antilymphocytic sera (ALS) between human normal and cancer cells, (5) Initiation of studies on cell fusion, hybridization and keterokaryon formation *in vitro*, and (6) detection of gs antigen in human embryonic tissue and tumor cells.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all SVCP contractors and Viral Oncology intramural staff. All of the most important viral specimens, cell cultures, sera, etc., used in the Viral Oncology Program are sent to this facility for PPLO testing and many of the SVCP contractors are dependent upon this facility for this service or as a check on their own technics. In addition the contractor is growing human tumor cells *in vitro* as a resource for other SVCP contractors and for the purpose of his own research on the detection and/or isolation of a human cancer virus or oncogene.

Date Contract Initiated: June 19, 1969

Current Contract Level: $175,000

WEIZMANN INSTITUTE OF SCIENCE (NIH 69-2014)

Title: Study of Virus-Induced Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Charles W. Boone

Objectives: (1) To investigate the differences between the structure of the surface membranes of normal cells and of cells transformed by viral and non-viral carcinogens; (2) to determine oncogenic virus-specific changes in the structure of cell surface membrane by studying the binding of amino acid co-polymers and carbohydrate binding agglutinins.

Major Findings: It was shown that a certain plant glycoprotein agglutinates mouse, rat, and human cell lines transformed by oncogenic viruses but not normal cells from which the transformed cells were derived. The agglutination was specifically inhibited by a number of saccharides. The binding sites of the glycoprotein were found to exist in cryptic form in normal cells and could be exposed by treatment with trypsin. The size and conformation of the binding site was studied. In addition ornithine-leucine co-polymer was found to produce a specific aggregation of SV40 transformed cells.

Significance to Biomedical Research and the Program of the Institute: Current research in tumor immunology has shown that a large number of human cancers have tumor-specific antigens against which the cancer patient has mounted an immune response. This contract is aimed at understanding and characterizing virus-induced tumor-specific transplantation antigens similar in nature to those found in humans. Information derived from these studies will be useful in determining how human tumor specific antigens can be used both diagnostically and therapeutically.

Proposed Course: The research will continue to be concerned with the use of purified carbohydrate-binding proteins and glycoproteins and synthetic polyamino acids in order to investigate the structural difference in the surface membrane between normal cells and transformed cells, with particular reference to virus-induced transformation.

Date Contract Initiated: April 22, 1969

Current Contract Level: $50,000

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NIH 71-2092)

Title: Extraction and Characterization of Virus-Induced Transplantation Antigen from Sarcomas and Leukemias

Contractor's Project Director: Dr. Anthony J. Girardi

Project Officer (NCI): Dr. George J. Todaro

Objectives: To extract and characterize induced tumor-specific transplantation antigens of selected DNA and RNA tumor viruses.

Major Findings: Studies on enzymatic release of SV40 TSTA from hamster tumor cells have indicated the specificity of the reaction in that positive activity was released from SV40 transformed hamster cells but not from cells transformed by either adenovirus type 7, Rous sarcoma virus, or murine sarcoma virus. A mouse cell, transformed by SV40, but not demonstrating SV40 TSTA activity even when intact, did not release the active antigen. Concentration of the antigen was not achieved by batch Sephadex treatment and studies are in progress employing differential centrifugation, diaflo membranes, and vacuum dialysis.

The use of neuraminidase resulted in release of SV40 TSTA from lytically infected AGMK cells harvested 21 hours post infection. By this technic it may be possible to determine the time of expression of this antigen during the lytic cycle.

Microsomal lipoprotein extracts of SV40 transformed hamster cells did not contain SV40 TSTA activity even when employed in hamsters receiving complete Freund's adjuvant.

Standard antigen and antiserum for use in agar immunodiffusion studies have been prepared. These are being employed for studying the development of tumor resistance *in vivo* and for evaluation of vaccines *in vitro*.

BUDR treatment of SV40 transformed hamster cells did not result in suppression of the SV40 TSTA although it had previously been shown that SV40 T antigen expression had been greatly diminished.

Irradiation at 20,000 r did not appreciably alter SV40 TSTA activity. Some destruction was noted at 50,000 r, although it was minimal.

Transplantation antigen studies with both Rauscher leukemia and murine sarcoma have yielded poor results regardless of whether intact cells, extracts, or infectious virus was employed as experimental vaccines. Even where murine sarcomas were either suppressed (or where regressions occurred) the survivors died subsequently with leukemia.

Significance to Biomedical Research and the Program of the Institute: Current research in tumor immunology is showing that human tumors have tumor-specific antigens which induce a cellular immune response in the host. Understanding and utilizing this immune cancer involves the isolation and characterization of tumor-specific transplantation antigens.

Proposed Course: (1) Continue studies on the preparation of cell membranes and extracts from immunogenic, SV40 virus-induced tumor development, (2) continue investigations on presence of immunogenically significant transplantation antigen in RNA virus-induced malignancies, and (3) to determine whether murine and feline leukemia are related to human leukemia as determined by shared transplantation antigens.

Date Contract Initiated: February 1, 1971

Current Contract Level: $60,000

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY (NIH 69-2007)

Title: Isolation and Purification of Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Donald F. H. Wallach

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To investigate the biochemical and antigenic changes which occur in the plasma membranes of cells during and after transformation by oncogenic viruses.

Major Findings: During the last year numerous cell lines were tested for optimum growth of SV40 virus. Assays to monitor virus infection were set up and a novel culture method was devised for easily growing large quantities of mammalian cells. Specialized electrophoretic technics using both gel slabs and gradient gels were developed. Most significantly, a "neoprotein" was found to be present in the membranes of cells permissively infected with SV40 virus. In addition, the development of a theoretical basis for ultracentrifugal fractionation, in conjunction with applied liquid-shear technics for the fragmentation of membranes, demonstrated utility not only for the separation of plasma membranes from other membrane classes, but also for the subfractionation of membrane fragments into different functional and topological entities. A method for the spectrophotofluorometric analysis of polyoma and SV40 T-antigens showed promise in the analysis of membrane protein changes during permissive infections or neoplastic conversions with oncogenic DNA viruses.

Significance to Biomedical Research and the Program of the Institute: The characterization of structural and functional properties of tumor cell membranes using virus-induced tumor antigens as a model, promotes the development of powerful biochemical and immunological tools for the diagnosis and treatment of human tumors.

Proposed Course: To continue to investigate the biochemical and antigenic changes which occur in the plasma membranes of cells during and after transformation by oncogenic viruses.

Date Contract Initiated: March 12, 1969

Current Contract Level: $40,700

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TABLE OF RATIOS OF CONTRACT TO INTRAMURAL EFFORT BASED ON CURRENT LEVEL OF EXPENDITURE
JUNE 30, 1971

	OSDE	Demography	Carcinogenesis	Viral Oncology	Total
Total	1,121,000	5,613,000	12,938,000	36,050,000	55,722,000
Contracts	--	4,033,000	10,598,000[2]	31,873,000[4]	46,504,000
Intramural*	1,121,000[1]	1,580,000	2,340,000[3]	4,177,000[5]	9,218,000
Ratios: Contract to Intramural	--	2.5:1	4.5:1	7.6:1	5.0:1

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*Includes costs of managing contract activities.

[1] Includes $564,000 for data processing for all Branches.

[2] Includes $896,000 for Lung Cancer.

[3] Includes $119,000 for Lung Cancer.

[4] Includes $234,000 funded by Breast Cancer Task Force and excludes $100,000 for GL&C contract program.

[5] Includes $294,000 contract funds for EVIF.

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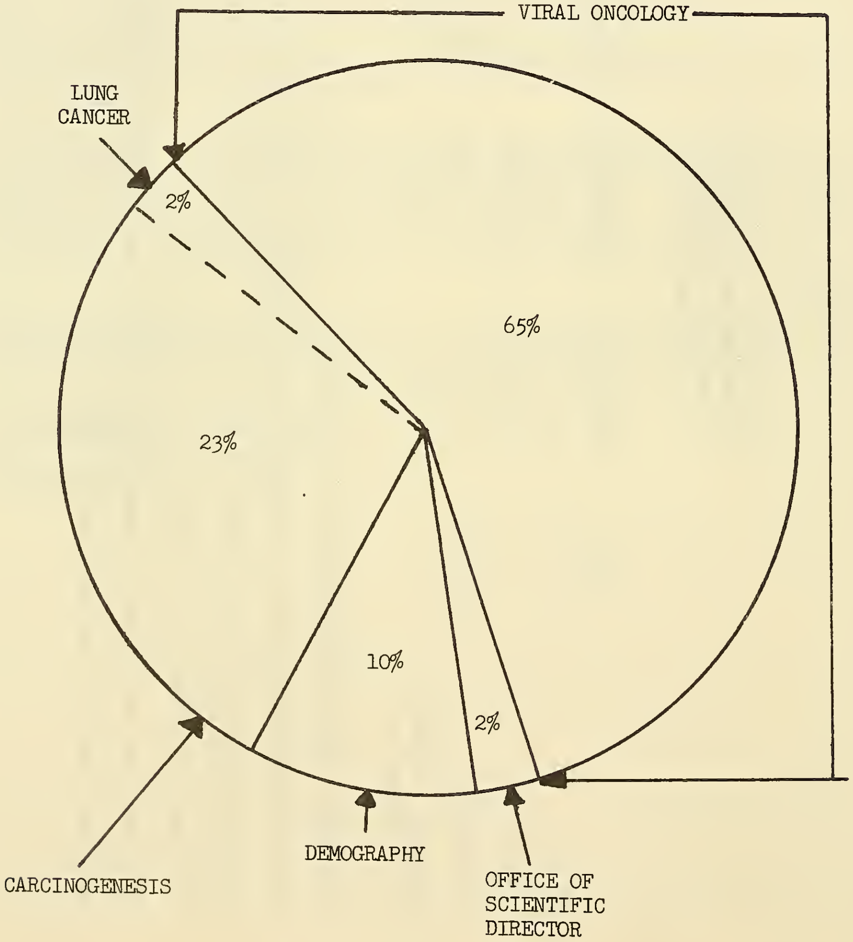
CURRENT DISTRIBUTION OF FUNDS-FISCAL YEAR 1971



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Special Cancer Studies Section

Miller, Robert W.	Acting Head

Office of Associate Scientific Director for Carcinogenesis

Saffiotti, Umberto	Associate Scientific Director
Glassman, Morris I.	SA Surgeon
Heim, Allen H.	Res. Microbiologist
Newell, Raymond F.	Supv. Gen. Engineer

Lung Cancer Unit

Sporn, Michael B.	SR Surgeon, Head
Al-Arif, Adhid	Visiting Fellow
DeLuca, Luigi M.	Res. Chemist
Harris, Curtis	SA Surgeon
Kaufman, David	SA Surgeon

Epidemiologic Pathology Unit

Berg, John W.	Medical Officer, Head
Lingeman, Carolyn H.	Medical Officer
Feldman, Henry A.	SA Health Services Officer

Program and Data Analysis Unit

Cooper, John A., II	Res. Chemist, Head
Page, Norbert P.	Vet. Officer
Rosenberg, Howard	SA Sanitary Engineer

Biology Branch

Office of the Chief

Rapp, Herbert R.	Chief
Bartlett, Gerald L.	SA Surgeon

Molecular Separation Unit

Rapp, Herbert R.	Acting Head
Stewart, Laura C.	Res. Chemist

Biology Branch (cont'd)

Cellular Immunity Section

Zbar, Berton	SR Surgeon, Head
Bernstein, Irwin D.	SA Surgeon

Cytogenetics & Cytology Section

DiPaolo, Joseph	Res. Pharmacologist, Head
Oshiro, Yuki	Staff Fellow

Immunochemistry Section

Borsos, Tibor	Res. Chemist, Head
Leonard, Edward J.	Medical Director
Sassano, Felix G.	Surgeon
Wyatt, Harold V.	Visiting Scientist

Chemistry Branch

Office of the Chief

Gelboin, Harry V.	Chief

Cell Growth Regulation Section

Bader, John P.	Res. Microbiologist, Head
Clark, Joseph M.	Surgeon
Hatfield, Dolph	Res. Biologist
Kitano, Yoshiyuki	Visiting Fellow
Polacow, Ione	Visiting Fellow

Molecular Carcinogenesis Section

Gelboin, Harry V.	Acting Head
Kinoshita, Nadao	Visiting Scientist
Salomon, Raphael	Visiting Fellow
Wiebel, Friedrich	Biochemist

Nucleic Acids Section

Dingman, C. Wesley	Medical Officer, Head
Brown, Richard J.	Surgeon
Kakefuda, Tsuyoshi	Medical Officer
Swann, Peter F.	Visiting Fellow

Protein Section

Peacock, Andrew C.	Res. Chemist, Head
Green, Marie R.	Res. Chemist
Ness, Arthur T.	Res. Chemist
Zeiger, Robert S.	SA Surgeon

Experimental Pathology Branch

Office of the Chief

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Histopathology Unit

Bates, Richard R.	Medical Director, Acting Head

Bioassay Section

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Rice, Jerry M.	Scientist
Hennings, Henry	Staff Fellow

Carcinogen Screening Section

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Weisburger, Elizabeth K.	Scientist Director
Williams, Gary M.	SA Surgeon
Yamamoto, Richard S.	Res. Chemist
Grantham, Preston H.	Res. Chemist
Giao, Nguyen-Ba	Visiting Scientist

Office of Associate Scientific Director for Viral Oncology

Moloney, J. B.	Associate Scientific Director
Sibal, Louis R.	Microbiologist
Burton, George J.	Scientist Director
Rubin, Daniel J.	Staff Fellow
Kvedar, John P.	Bio. Technologist

Office of Biohazards & Environmental Control

Hellman, Alfred	Scientist Director, Head

Biohazards Research Section

Hellman, Alfred	Scientist Director, Head
Steinman, Harry G.	Supv. Chemist
Fowler, Arnold K.	SR Scientist

Environmental Control Section

Barkley, William E.	San. Engineer, Acting Head
West, David L.	A San. Engineer

Office of the Coordinator of Ultrastructural Studies

Dalton, Albert J.	Coord. of Ultrastructural Studies

Virus Studies Section

Heine, Ursula I.	Res. Microbiologist, Head
Suskind, R. Gerald	Medical Director
Bader, Artrice V.	Research Biologist

Office of Program Analysis & Communications

Waggoner, Deward E.	Statistician, Head
Greenberg, Louis P.	Tech. Information Specialist
Varrato, Wilma L.	Statistical Assistant

Viral Biology Branch

Office of the Chief

Manaker, Robert A.	Supv. Res. Micro., Acting Chief
Chirigos, Michael A.	Res. Chemist, Associate Chief
O'Connor, Timothy E.	Head, Molecular Virology
Reisinger, Robert C.	Veterinarian
Gruber, Jack	Microbiologist

Cell Biology Section

Boone, Charles W.	Medical Officer, Head
Gail, Mitchell H.	SA Surgeon
Koegel, Robert J.	Res. Chemist

Electron Microscopy Section

Chopra, Harish C.	Sr. Staff Fellow, Acting Head

Training and Service Unit

Lloyd, Bolivar J., Jr.	Biologist, Head
Mitchell, Elisabeth Z.	Chemist

Experimental Pathology Section

Manaker, Robert A.	Acting Head

Human Tumor Studies Section

Dunkel, Virginia C.	Sr. Staff Fellow, Acting Head
Ebert, Paul S.	Res. Chemist
Margolis, Sam A.	Sr. Staff Fellow
Pearson, Gary R.	Sr. Staff Fellow

Viral Biology Branch (cont'd)

Microbiology Section

Turner, Willie	Res. Microbiologist, Acting Head

Virus and Disease Modification Section

Chirigos, Michael A.	Res. Chemist, Head
Woods, Wilna A.	Res. Microbiologist
Pearson, John W.	Sr. Staff Fellow
Schwartz, Donald B.	SA Surgeon

Viral Carcinogenesis Branch

Office of the Chief

Huebner, Robert J.	Med. Director, Chief
Duff, James T.	Assoc. Chief
Zeve, Victor H.	Res. Biologist
Kelloff, Gary J.	Sr. Staff Fellow
Streicher, Harriet L.	Program Assistant
Shiflett, Shirley B.	Statistical Asst.
Capps, Worth I.	Bio. Lab. Tech.

Ecology and Epizoology Section

Sarma, Padman S.	Res. Microbiologist, Head

Field Studies Unit

Arnstein, Paul	Vet. O. Dir.
Estes, John D.	Bio. Lab. Tech.

Solid Tumor Virus Section

Duff, James T.	Res. Microbiologist, Head
Hampar, Berge	Sr. Dental Surgeon

Viral Genetics Section

Huebner, Robert J.	Acting Head
Portugal, Franklin H.	Sr. Staff Fellow
Parks, Wade P.	SA Surgeon
Simonds, Josephine	Staff Fellow

Serology Unit

Turner, Horace C.	Supv. Bio. Lab. Tech.

Trailer Unit

Lane, William T.	Bio. Lab. Tech.

Viral Leukemia and Lymphoma Branch

Office of the Chief

Todaro, George J.	Medical Officer, Chief
Holdenried, Robert	Scientist Dir., Assoc. Chief
Leiseca, Sergio A.	Research Biologist
Levine, Paul H.	Medical Officer
Kinard, Roy F., Jr.	Vet. O. Dir.
Sherer, David M.	Surgeon
Perkins, Ida V.	Microbiologist

Genetics Section

Todaro, George J.	Acting Head
Scher, Charles D.	Surgeon
Ablashi, Dharam V.	Res. Microbiologist

Immunology Section

Hollis, Vincent W., Jr.	Res. Chemist, Acting Head
Gerwin, Brenda I.	Sr. Staff Fellow
Plata, E. J.	Sr. Staff Fellow
Meltzer, Monte S.	SA Surgeon

Molecular Biology Section

Aaronson, Stuart A.	Medical Officer, Head
Scolnick, Edward M.	Sr. Staff Fellow
Ross, Jeffrey	SA Surgeon

Tumor Virus Section

Fischinger, Peter J.	Medical Officer, Head
Bassin, Robert H.	Sr. Staff Fellow, Acting Head
Phillips, Leo A.	Sr. Staff Fellow
Haapala, Daniel K.	Staff Fellow
Nomura, Shigeko	Res. Biologist

Viral Pathology Section

Gazdar, Adi F.	Medical Officer, Head
Merwin, Ruth M.	Res. Biologist
Easton, John M.	Sr. Surgeon
Peebles, Paul T.	EIS Officer

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